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Identification of a broad-specificity nucleoside transporter with affinity for the sugar moiety in *Giardia intestinalis* trophozoites

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A broad-specificity nucleoside transporter has been identified in *Giardia intestinalis* trophozoites, using a rapid sampling assay to measure influx of [^{3}H]deoxycytidine, [^{3}H]adenosine and [^{3}H]guanosine at 0°C. The influx of each labelled nucleoside was inhibited strongly by all common, naturally-occurring nucleosides but only poorly or not at all by nucleobases, indicating that the transporter recognizes structural features on the furanosyl moiety of ribo- and 2'-deoxyribonucleosides. Both 2'- and 5'-deoxyadenosine were potent inhibitors of influx (> 95% inhibition at 2 mM), whereas 3'-deoxyadenosine was significantly less effective (approx. 70% inhibition), and 2',3'-dideoxycytidine and cytosine arabinoside were virtually inactive (0–20% inhibition). The data reveal that the 2'- and 5'-hydroxyl groups are not necessary for the recognition of nucleosides by this transporter. However, the 3'-hydroxyl appears to be important. Michaelis-Menten constants (K_m) were calculated for the influx at 0°C of deoxycytidine (220 ± 116 μM) and adenosine (45 ± 24 μM), with respective V_{max} values of 13 ± 4 and 11 ± 2 pmol min^{-1} (10^6 cells)$^{-1}$. Only 12–26% of [^{3}H]thymidine influx occurred through this transporter, the remainder entering the cells through a thymine/uracil-specific transporter described previously. Thymidine exhibited a K_i of 205 ± 90 μM against [^{3}H]deoxycytidine influx.

Introduction

Giardia intestinalis (syn. *G. lamblia*) is an aerotolerant, anaerobic protozoan which colonizes the human small intestine [1,2]. It has been shown with axenically cultured trophozoites of two independent isolates (WB and Portland-1) that the cells lack the enzymes necessary for de novo synthesis of purines [3] and pyrimidines [4–6] and it is probable that the organism relies on exogenous purine and pyrimidine bases or nucleosides to satisfy its nucleotide requirements. There is little or no direct utilization of ribonucleosides. These are hydroiysed intracellularly to uracil, adenine and guanine, which are then converted to the respective 5'-mononucleotides by specific phosphoribosyltransferases [6–10]. *G. intestinalis* also appears to lack ribonucleotide reductase, the enzyme responsible in most organisms for the conversion of ribonucleotides to 2'-deoxyribonucleotides [11]. Because 2'-deoxyribose apparently is not utilised [10], it is likely that the parasite can synthesize deoxyribonucleotides only by direct phosphorylation of exogenously-acquired deoxynucleosides [6,10,11].

The membrane transport systems that must exist for cellular uptake of nucleosides and nucleobases by *Giardia* trophozoites remain essentially uncharacterized. Recently, we described a transporter of thymidine that is inhibited specifically by thymine, uracil, deoxyuridine and thymidine, but not by cytosine, cytidine or deoxycytidine [12]. This transporter is, thus, specific for uracil/thymine derivatives, and one may expect it to mediate the transport of uridine as well as thymidine. Thymidine influx was measured at 0°C to prevent adherence of trophozoites to vessel walls and the uptake of radiolabel at this temperature was shown to reflect membrane transport with minimal enzymic metabolism of thymidine. Previous studies, in which uptake was measured at 37°C [13,14], were found to reflect metabolism because of the rapid conversion of labelled bases into phosphorylated derivatives.

Because deoxycytidine, probably an essential nutrient, did not inhibit the thymine/uracil-specific thymidine transporter, we have examined the influx of this nucleoside, and that of adenosine and guanosine, into trophozoites of the Australian *G. intestinalis* isolate, Ad-1. This has led to the identification of a second nucleoside transporter that recognizes the β-furanosyl

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moiety of purine and pyrimidine ribonucleosides and 2'-deoxynucleosides.

Materials and Methods

Giardia cultures

G. intestinalis trophozoites (Adelaide-1 isolate, Ref. 15) were grown axenically as described previously [12]. Adherent cells were harvested and washed 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 $(1–1.6) \cdot 10^8$ cells ml^{-1} in ice-cold assay buffer (20 mM D-glucose, 2 mM L-cysteine-HCl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$ in PBS_m) containing 15 mg ml^{-1} bovine serum albumin and were used within 1 h.

[^{3}H]-Nucleoside stock solutions

A 2×-concentrated solution containing [5-^{3}H]deoxycytidine (Amersham TRK.211; 833 GBq $mmol^{-1}$), [2-^{3}H]adenosine (Amersham TRK.423; 962 GBq $mmol^{-1}$), [*methyl*-^{3}H]thymidine (Amersham TRK.300; 925 GBq $mmol^{-1}$) or [8-^{3}H]guanosine (Sigma 32217–2; 130 GBq $mmol^{-1}$), unlabelled carrier nucleoside (sufficient to bring the total nucleoside concentration to 10 μM) and 5 mg ml^{-1} (1-^{14}C)-acetylated bovine serum albumin [12] was prepared in assay buffer. In inhibition studies, the concentration of unlabelled nucleoside was varied or competitors were included. The purity of all radiolabelled nucleosides was verified by thin-layer chromatography (see below). Only the [^{3}H]guanosine required purification prior to use.

Rapid sampling assay

Uptake and transport at 0–4°C was measured as described [12,16]. Briefly, trophozoites were mixed with an equal volume of 2×-concentrated [^{3}H]nucleoside/[^{14}C]albumin solution and incubated on ice, usually for 40 to 60 s as indicated. 10 s prior to the end of the uptake period, a 50 μl sample ($(2.5–4.0) \cdot 10^6$ cells) was layered over a 230 μl oil phase and the cells were then sedimented by microcentrifugation 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 was calculated by measuring the [^{14}C]albumin content of each underlay.

Intracellular metabolism of transported [^{3}H]deoxycytidine

After various periods of incubation at 0°C with [^{3}H]deoxycytidine, trophozoites were sedimented into an underlay containing cytosine, cytidine, 2'-deoxycytidine, uracil, uridine and 2'-deoxyuridine (each 0.8 mM) in 1 M KOH. The recovered underlay was neutralised with $HClO_4$ and the labelled metabolites and added standards were separated by thin-layer chromatography on silica gel sheets (Kieselgel 60 F_{254}, Merck, Darmstadt) in two solvent systems. Butanol/acetic acid/H_2O (5:1:3, v/v) was used to separate possible deamination products of deoxycytidine (uridine, R_F 0.45; uracil + deoxyuridine, R_F 0.55) from cytosine, cytidine and deoxycytidine (R_F 0.22), while a butanol:aqueous ammonia (15 M)/H_2O (6:1:2, v/v) system separated bases from nucleosides and deoxynucleosides (R_F 0.34, 0.10 and 0.24, respectively). Phosphorylated products were immobile in both systems. The extent of metabolism of the transported deoxycytidine was deduced from the combined chromatographic results. Metabolism of [^{3}H]adenosine was studied using a protocol identical to that for deoxycytidine but with adenine and adenosine markers.

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 ^{3}H and ^{14}C channel separation and quench monitoring by 'H' number. Calculations utilized the measured ratio of ^{3}H to ^{14}C in each assay mixture to determine the extracellular ^{3}H content, which was then subtracted from the total cell-associated ^{3}H content.

Analysis of transport kinetics

(a) Calculation of K_m and K_i. Transport was considered to be a saturable process following Michaelis-Menten kinetics [17,18]. Influx was measured in duplicate or triplicate tubes over a 40 s (adenosine) or 60 s (deoxycytidine) period at 0°C to obtain near-initial velocities. The Michaelis-Menten constants (K_m) for adenosine and deoxycytidine influx were calculated by fitting the data to rectangular hyperbolas by non-linear regression using both EZ-FIT (F.W. Perrella, DuPont de Nemours, 1988) and Inplot (V3.1; GraphPAD Software, San Diego, CA). K_i values for thymidine were calculated from Dixon plots [19].

(b) Calculation of relative inhibitor constants. The concentration (IC_{50}) of various nucleosides and nucleobases required to halve the influx of 5 μM deoxycytidine over 1 min at 0°C was calculated by measuring uptake of [^{3}H]deoxycytidine over a range of inhibitor concentrations (4–1700 μM). Rectangular hyperbolas were fitted to each data set by non-linear regression.

(c) Inhibition of thymidine transport by uracil and deoxycytidine. The influx of thymidine through the thymine/uracil-specific thymidine transporter [12] and through the broad-specificity nucleoside transporter

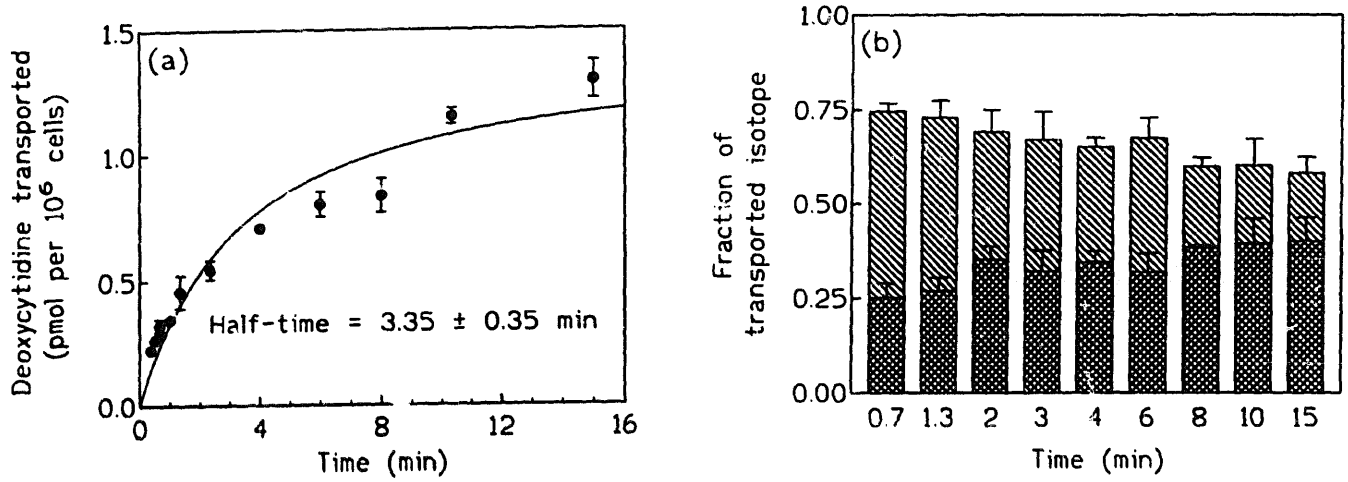

Fig. 1. (a) Time course of deoxycytidine uptake into trophozoites at 0°C. Cells were incubated with [^{3}H]deoxycytidine for the indicated times (triplicates) and then sedimented without dilution through oil. (b) Intracellular metabolism of transported [^{3}H]deoxycytidine at 0°C. Phosphorylated, deaminated and hydrolysed products were monitored in trophozoites over time. Thin-layer chromatography indicated that label was associated only with deoxycytidine and uracil. Incorporation of label into nucleotides was below the level of detection. The fraction of intracellular label (mean ± 1 S.D.) present as deoxycytidine (single + double hatched) or uracil (double hatched) is shown.

was examined by incubating trophozoites with [^{3}H]thymidine in the presence of unlabelled thymidine, uracil, or deoxycytidine (each at a final concentration of 1.5 mM) for periods of up to 10 min at 0°C.

Results

Measurement of 2'-deoxycytidine uptake

Uptake of [^{3}H]deoxycytidine at 0°C was monitored over periods up to 15 min (Fig. 1a). Accumulation of deoxycytidine to a calculated equilibrium level of 1.4 ± 0.1 pmol(10^6 cells)$^{-1}$ was evident, with half this level being reached by 3.4 ± 0.4 min. Taking into consideration the amount of label needed for accurate measurements and the need for estimates of initial influx rates, a 1-min fixed-time uptake period was chosen for subsequent kinetic experiments. At this time, influx was still essentially linear, with mean influx rates 85 ± 5% of initial rates calculated by extrapolation from timed measurements. The uptake was saturable, as it was inhibited by unlabelled deoxycytidine, as exemplified in Fig. 2. Combined data from this and two other independent experiments, using concentrations of deoxycytidine up to 1 mM, yielded K_m 220 ± 116 mM (mean ± S.D.) and V_{max} 13 ± 4 pmol transported min^{-1}(10^6 cells)$^{-1}$.

Intracellular metabolism of deoxycytidine

Trophozoite lysates contain substantial cytidine deaminase activity, but direct hydrolysis of cytidine to cytosine is insignificant [4,5]. Rapid intracellular metabolism of labelled deoxycytidine, especially phosphorylation, could have complicated the interpretation of uptake kinetics seriously. The products of phosphorylation, deamination and hydrolysis were therefore measured by thin-layer chromatography after various

TABLE I

Inhibition of [^{3}H]deoxycytidine influx into trophozoites by nucleobases and nucleosides

The influx of [^{3}H]deoxycytidine into trophozoites was measured over 1 min at 0°C in the presence of different unlabelled nucleosides or nucleobases as competitors, each tested at 4–5 concentrations. The data (from at least two separate experiments for each competitor) were used to calculate the mean concentration (IC_{50} ± 1 S.E.) required to halve the rate of uninhibited influx.

Base substituent	IC_{50} (μM)				
	adenine	cytosine	guanine	thymine	uracil
Free base	>1000	>1000	>1000	>1000	>1000
Ribonucleoside	25 ± 5	92 ± 13	26 ± 5	– [a]	45 ± 25
2'-Deoxynucleoside	89 ± 8	96 ± 5	93 ± 13	115 ± 5	93 ± 13
3'-Deoxynucleoside	550 ± 100	– [a]	– [a]	–	– [a]
5'-Deoxynucleoside	40 ± 20	–	–	–	–
2',3'-Dideoxynucleoside	– [a]	>1000	–	–	–
Arabinoside	–	>1000	–	–	–

[a] Not tested.

periods of uptake by trophozoites at 0°C (Fig. 1b). After 1 min of uptake, intact deoxycytidine represented at least 73% of the cell-associated label. All of the remaining label co-migrated with uracil, indicating that deamination was rate-limiting and that hydrolysis of deoxyuridine [7] was rapid. Phosphorylation and conversion to deoxyuridine, uridine and cytosine were below the level of detection for the entire time course (up to 15 min). These results showed that measurements of deoxycytidine uptake over 1 min at 0°C reflected influx, not metabolism.

Specificity of the deoxycytidine transporter

The inhibition of 2'-deoxycytidine influx by a range of purine and pyrimidine bases, nucleosides and deoxynucleosides was measured (Table I). Ribonucleosides were the best competitors, with adenosine, guanosine and uridine having IC_{50} values of 25–45 μM. The 2'-deoxynucleosides (IC_{50} range 89–115 μM) appeared slightly less effective. The free bases were extremely poor competitors ($IC_{50} > 1$ mM), indicating that the transporter requires the presence of the sugar moiety for recognition of nucleosides. The inhibitory capacity (IC_{50}) of 5'-deoxyadenosine (40 ± 20 μM) was similar to that of adenosine (25 ± 5 μM), but 3'-deoxyadenosine was significantly less active (550 ± 100 μM). Cytosine-arabinoside and 2',3'-dideoxycytidine had little inhibitory activity ($IC_{50} > 1$ mM). In addition to the competitors listed in Table I, nitrobenzyl-mercaptopurine riboside (NBMPR) and dipyridamole (both potent inhibitors of mammalian nucleoside transport) were tested near the limit of their solubilities (from 0.02 to 20 μM). NBMPR was not inhibitory and dipyridamole inhibited by < 10% at the highest concentration used (data not shown).

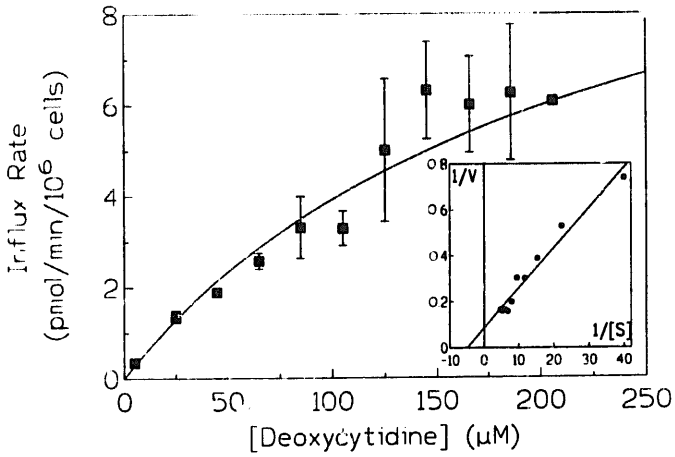

Fig. 2. Plot of deoxycytidine influx velocity (pmol min^{-1} (10^6 cells)$^{-1}$) vs. deoxycytidine concentration, using data from one of 3 separate experiments. Uptake measurements were 1 min at 0°C. (Inset) Double reciprocal plot of the same data ($[S]^{-1}$ values in mM^{-1}), with the 5 mM datum point omitted to visualize the distribution of the other points.

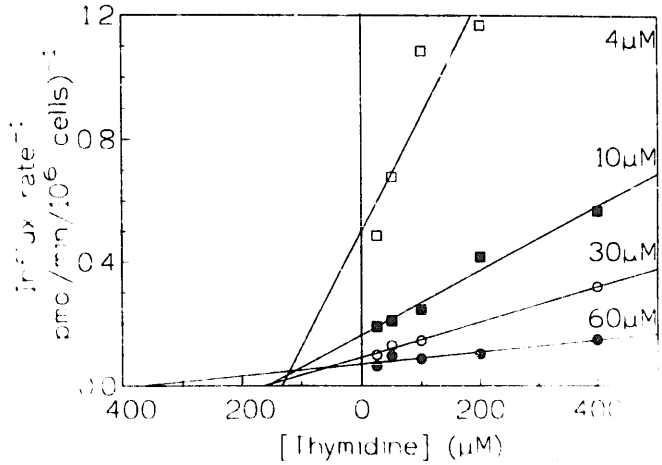

Fig. 3. Dixon plot depicting the inhibition of deoxycytidine influx into trophozoites by thymidine. The graph depicts the reciprocal initial velocities (pmol min^{-1} (10^6 cells)$^{-1}$) of deoxycytidine influx measured over a range of thymidine concentrations at four different concentrations (4 to 60 μM) of deoxycytidine (indicated) from one of two separate experiments. Each point represents the mean of duplicate samples. Lines were fitted by least-squares analysis. The point of intersection of each pair of lines was used to calculate a mean value ($-K_i$).

Inhibition of deoxycytidine influx by thymidine

Because thymidine enters trophozoites via a high-affinity, base-specific transporter [12], we examined the inhibition of [^{3}H]deoxycytidine influx by thymidine. Uracil, which does not inhibit deoxycytidine transport, was used to minimise any interference that might have arisen by thymidine entering the cells through the thymine/uracil-specific thymidine transporter. Dixon plot analysis of the kinetic data (Fig. 3) indicated that thymidine inhibited deoxycytidine transport in a simple competitive manner, with a mean K_i (±S.E.) of 205 ± 90 μM (calculated from two experiments).

Specificity of adenosine and guanosine uptake

Time course studies of adenosine uptake showed that this nucleoside entered trophozoites faster at 0°C than did deoxycytidine, half the equilibrium concentration being reached by 2.42 ± 0.38 min (data not shown). Chromatographic analysis of intracellular metabolites after 40 s uptake at 0°C with 5 μM [^{3}H]adenosine (≧ 98% pure) revealed that 63% had been hydrolyzed to adenine, ≦ 3% to phosphorylated derivatives, with 34% remaining intact (data not shown). This substantial rate of metabolic catabolism, significantly faster than observed with deoxycytidine, was consistent with the known high level of adenosine hydrolase in *G. intestinalis* trophozoites [3,14].

To determine whether a single broad-specificity transporter was responsible for the influx of deoxycytidine, adenosine and other non-thymine-based nucleosides in *G. intestinalis*, the uptake of labelled adenosine and guanosine was examined over 45 s at 0°C in the

presence of various competitors (Table II). The entry of each labelled nucleoside was inhibited severely (78–98%) by all of the nucleosides examined, except 3'-deoxyadenosine (approx. 70% inhibition) and 2,3-dideoxycytidine (15–24%). This pattern of inhibition is identical to that observed for deoxycytidine influx. Moreover, the pattern cannot be explained by inhibition of the adenosine-guanosine hydrolase, because uridine, cytidine and thymidine (inhibiting uptake by 78 to 96%) have no effect, even at 2 mM, on the activity of this enzyme (Ey, P.L. and Andrews, R.H., unpublished data). These uptake data therefore reflect predominantly transport kinetics. A single competition experiment using unlabelled adenosine yielded a Michaelis-Menten constant for adenosine uptake of 45 ± 24 μM, with V_{max} 11 ± 2 pmol min^{-1} $(10^6$ cells$)^{-1}$ (data not shown).

Contribution of the broad-specificity nucleoside transporter to thymidine influx

The contribution of the newly defined transporter to the transport of labelled thymidine (external concentration, 4.8 μM) into trophozoites was examined in the presence of 1.5 mM unlabelled bases or nucleosides (a 300-fold molar excess; Fig. 4). Relative to the maximally-inhibited rate observed in the presence of 1.5 mM unlabelled thymidine (which should inhibit both nucleoside transporters), uracil inhibited the rate of influx by 79–88%, and deoxycytidine by only 19–26%. As discussed earlier, uracil inhibits the thymine/uracil-specific thymidine transporter but not the broad-specifity nucleoside transporter, whereas deoxycytidine has the reverse effect. The effect of both competitors (each 1.5 mM) was essentially additive, showing that thymidine entered the cells predominantly (but not exclusively) through the base-specific transporter. The extrapolated steady-state level for uninhibited thymidine influx was 1.4 ± 0.2 pmol $(10^6$ cells$)^{-1}$, identical to that calculated for [^{3}H]deoxycytidine.

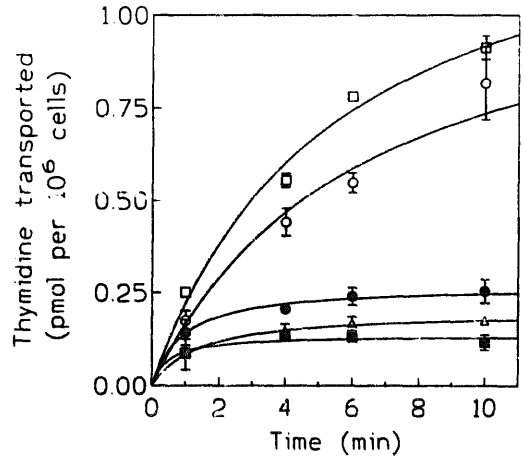

Fig. 4. Inhibition of thymidine influx into trophozoites. Deoxycytidine, uracil and a combination of both (each 1.5 mM) were tested for their capacity to inhibit the rate of thymidine influx, relative to thymidine itself (1.5 mM, △). □, uninhibited influx. Deoxycytidine (○) inhibited the rate of influx by 19–26%, whilst uracil (●) reduced the rate by 79–88%. The efficiacy of uracil plus deoxycytidine (■) was not significantly different from that of thymidine.

TABLE II

Inhibition of adenosine and guanosine uptake

The uptake of [^{3}H]adenosine and [^{3}H]guanosine by trophozoites was measured over 45 s at 0°C using 5 μM [^{3}H]nucleoside in the presence or absence of different unlabelled competitors, each at a final concentration of 2 mM, except where indicated.

Unlabelled competitor (2 mM)	% Inhibition of uptake	
	[^{3}H]adenosine	[^{3}H]guanosine
None	0±3	0±3
Adenosine	98±1	94±1
Inosine	97±1	96±1 [a]
Guanosine	94±0 [a]	96±1 [a]
Cytidine	82±8	-
Uridine	96±1	93±6
2'-Deoxythymidine	78±3	85±1
2'-Deoxycytidine	86±1	95±4
2'-Deoxyadenosine	95±0	97±5
3'-Deoxyadenosine	69±3	74±2
5'-Deoxyadenosine	96±1	96±2
2',3'-Dideoxycytidine	24±1	15±4
Adenine	27±8	-
Hypoxanthine	1±4	4±7
Uracil	-2±7	-
Thymine	-1±3	-

[a] 1 mM competitor (final concentration).

Discussion

The results of this study show that *G. intestinalis* trophozoites absorb deoxycytidine, adenosine and guanosine via a previously undescribed membrane transporter. The uptake of these nucleosides has been examined under conditions where metabolism is minimal, as shown previously for thymidine influx by a thymine/uracil-specific thymidine transporter [12] and verified for deoxycytidine and adenosine in this study. The initial findings, based on deoxycytidine uptake at 0°C, showed that this transporter was different from the thymine/uracil-specific thymidine transporter. These conclusions were confirmed and extended using labelled adenosine and guanosine.

A single transporter was found to be responsible for the influx of each of the three labelled nucleosides examined. The transporter has affinity for both ribonucleosides and 2'-deoxyribonucleosides (purine and pyrimidine), with slight preference for purine ribonucleosides. This conclusion is based on the finding that, for all three nucleosides examined, the pattern of inhibition of influx by a range of purine and pyrimidine

bases and nucleosides was virtually identical. The importance of the β-furanosyl ring of the nucleoside was indicated by the reduced inhibitory capacity of 3′-deoxyadenosine, compared with that of 2′-deoxyadenosine and 5′-deoxyadenosine, and the lack of inhibition by 2′,3′-dideoxycytidine and cytosine arabinoside. The nucleoside 2′- and 5′-hydroxyls are therefore not essential, but the 3′-hydroxyl is important and loss of both the 2′- and 3′-hydroxyl groups prevents recognition as assessed by inhibitory activity. A change in the conformation of the furanoside, as is forced by the inversion of the 2′-hydroxyl in cytosine arabinoside, also abolishes recognition.

At present, it is difficult to determine what role the base moiety plays in the recognition of nucleosides by this transporter. Free bases were largely ineffective inhibitors of deoxycytidine and adenosine influx, in striking contrast to the potent inhibition (by thymine and uracil) seen previously for the base-specific thymidine transporter [12]. Nevertheless, it appears that the base moiety does participate in nucleoside recognition by the broad-specificity transporter. This is indicated by the slight but consistent differences in gross inhibition activity and IC_{50} values observed for nucleosides containing different bases. With all three labelled nucleosides, cytidine, deoxycytidine and thymidine were less effective inhibitors of influx than were uridine and the purine ribo-and 2′-deoxyribonucleosides.

There are clear differences between the *G. intestinalis* nucleoside transporter described in this study, and most mammalian facilitated-diffusion nucleoside transporters for which the common nitrogens of pyrimidines (N-1) and purines (N-9), together with the 5′ sugar hydroxyl, have been implicated in substrate recognition [20]. The *G. intestinalis* transporter may interact similarly with the pyrimidine N-1/N-3 and the purine N-9/N-7 atoms, which are in equivalent positions. However, the failure of the *Giardia* transporter to discriminate between natural nucleosides and 5′-deoxyadenosine distinguishes this transporter from those detected in mammals generally. Nevertheless, it is interesting to note that the human erythrocyte nucleoside transporter shares with the *G. intestinalis* transporter a requirement for the 3′-hydroxyl of nucleosides [21]. Both of these broad-specificity nucleoside transporters show no requirement for the 2′-hydroxyl moiety, but have significantly reduced affinity for 3′-deoxynucleosides and they appear unable to recognize 2′,3′-dideoxycytidine. Despite this remarkable similarity in the structural requirements for substrate recognition, these transporters can be distinguished on the basis of the high resistance of the *Giardia* transporter to nitrobenzyl-mercaptopurine riboside (NBMPR) and dipyridamole ($IC_{50} > 20$ μM). Both of these compounds are extremely effective inhibitors of facilitated nucleoside transport in mammals, including that of erythrocytes (IC_{50} range 0.001–0.1 μM) [18,22]. Although the mammalian and *Giardia* transporters share similar broad substrate affinity (K_m 100–300 μM) and specificity, the differences in drug susceptibility provide potential to design chemotherapeutic agents that are selective for either the parasite or host transporters.

In our previous study [12], we reported that trophozoites possess a thymine/uracil-specific thymidine transporter (K_m 50 μM at 0°C) but observed also that the rate of thymidine influx was inhibited by up to 20% by nucleosides such as adenosine. That result can now be explained by the findings of this study which show that, at an external concentration of 5 μM, approx. 80% of thymidine influx occurs via the base-specific thymidine transporter. The remainder is mediated by the broad-specificity nucleoside transporter. Although the thymine/uracil-specific thymidine transporter has affinity for thymine (K_i 30 μM at 0°C), we have shown in other studies [23] that it does not contribute significantly to the transport of the free base. Further characterization of both *Giardia* nucleoside transporters will require detailed kinetic analyses (e.g., Ref. 18) over a range of temperatures to determine their behaviour under physiological conditions. At present, it would appear that the base-specific carrier constitutes a high-affinity uptake system for thymidine (and presumably uridine) and that it may be important when thymidine is scarce. The broad-specificity transporter described in this study seems vital for the purine ribo- and deoxyribonucleoside and deoxycytidine requirements of *G. intestinalis*.

Acknowledgements

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