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In situ kinetic characterization of methylthioadenosine transport by the adenosine transporter (P_2) of the African *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense*

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Abstract

African trypanosomes are parasitic flagellates that live in the connective tissues of the host. Trypanosomes must obtain from their host adenine/adenosine and other nucleosides that can be salvaged through enzymatic cleavage. Methylthioadenosine (MTA) is a byproduct of polyamine metabolism, formed from the donation of an aminopropyl moiety by decarboxylated *S*-adenosylmethionine (dcAdoMet) to form spermidine. MTA is then cleaved phosphorolytically by MTA phosphorylase to methylthioribose-1-phosphate (MTR-1-P) and adenine. The uptake of MTA was compared with that of adenosine in two strains: *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense.* The K_m values for MTA and adenosine (with 5 mM inosine) transport by *T. b. brucei* were 1.4 and 0.175 mM, and the V_{max} values were 70 and 7.8 μmol/L/min, respectively. The K_m values for *T. b. rhodesiense* MTA and adenosine (with 5 mM inosine) transport were 1.2 and 0.11 mM, and the V_{max} values were 52.6 and 2.9 μmol/L/min, respectively. Since MTA was not competitive with either AdoMet (100 μM), inosine (100 μM), or the methionine precursor ketomethylthiobutyrate (100 μM), it appears that MTA enters through the P_2 (adenosine/adenine) transport site. From this study and our previous work, we determined that these organisms transport adenylated intermediates of methionine metabolism found in sera for purine salvage and as an ancillary source of methionine. The significant ability of African trypanosomes to transport MTA and related intermediates is an important consideration in the design and development of selective chemotherapeutic agents.

Keywords: African trypanosomes; Methionine synthesis; Nucleoside transport; Kinetic analysis

1. Introduction

The importance of adenosine transport, as well as the diverse nature of the family of adenosine transport proteins in eukaryotes, has been the subject of numerous reviews [1,2]. Many parasitic protozoa cannot synthesize purines *de novo* and must obtain them in some form from their host. The transport of adenosine by African trypanosomes has been characterized [3] as occurring through two distinct transporters. The P_1 site transports adenosine/inosine, and the P_2 site transports adenosine/adenine. The difference has been shown to be a matter of specificity and the quantitative amounts of adenosine that are transported through each port. These findings are in agreement with those from numerous other unicellular hemoflagellate parasites [2]. At present, the major drug being utilized for the treatment of human African trypanosomiasis is melarsoprol, the dimercaptoethanol derivative of melarsen oxide. Melarsoprol was shown by Yarlett *et al.* [4] to be lytic to susceptible, but not resistant stains. Using a lysis assay, it was later found to be transported through the P_2 adenosine transporter [5]. Carter and coworkers [3,5] also have shown that in certain laboratory-induced resistant strains of *Trypanosoma brucei brucei,* this transporter is missing or lowered in activity [activ-

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Abbreviations: AdoMet, *S*-adenosylmethionine; MTA, methylthioadenosine; MTR-1-P, methylthioribose-1-phosphate; KMTB, ketomethylthiobutyrate; HETA, 5′-deoxy-5′-hydroxyethylthioadenosine; and PBSG, 0.05 M phosphate-buffered 0.9% saline (pH 8.0) + 7% glucose.

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ity was described as having both lower affinity (K_m) and velocity (V_{max})]. Carter *et al.* [5] demonstrated that melarsen oxide resistance correlates with induced pentamidine resistance in a *T. b. brucei* laboratory strain. Barrett *et al.* [6] found comparable results for induced resistance with *Trypanosoma equiperdum.* It is well established [7] that pentamidine enters through the P_2 transporter. In the studies of Carter and Fairlamb [3], it was shown that transport through P_2 and $P_1 + P_2$ can be measured separately in the presence of millimolar concentrations of inosine, which will saturate the P_1 site.

To meet their methionine requirement, African trypanosomes have very active methionine salvage pathways [7,8]. AdoMet, after decarboxylation, donates an aminopropyl group for the synthesis of spermidine. MTA, the end product, is then recycled to methionine through a five-step pathway [9,10]. The first reaction in this pathway is catalyzed by MTA phosphorylase, which cleaves MTA to adenine and MTR-1-P. The MTR-1-P is converted through subsequent reactions to KMTB [9–11], which is transaminated to methionine utilizing aromatic amino acids as amine donors [7]. The ability to transport MTA or KMTB can thus help trypanosomes meet their methionine requirement, via this recycling pathway. It has been shown previously that MTA is present in human sera [12] and is taken up by both *Ochromonas malhamensis* and *Ochromonas danica,* protozoans used as models for human liver cell methionine metabolism [13,14]. In both of these studies, it was shown that use of MTA was an excellent way to conserve vitamin B_{12}, and contributes significantly to the methionine requirement of the organisms.

We have shown previously that AdoMet is taken up by trypanosomes through a unique transporter [15–17] and that it acts as a sink for methionine [15,18]. In those studies, we demonstrated that AdoMet results in the salvage of both methionine and adenosine. The majority of the AdoMet, which was utilized for transmethylation, was subsequently metabolized by the transsulfuration pathway following adenosine salvage by *S*-adenosylhomocysteine (AdoHcy) hydrolase [7]. In this study, we have extended the concept of adenine and methionine salvage in trypanosomes, demonstrating the transport of MTA, comparing it with the transport of adenosine and AdoMet, and examining the effects of intermediates of methionine and polyamine metabolism, and of HETA, a trypanocidal MTA analog, on uptake [19].

2. Materials and methods

2.1. Organisms and culture conditions

T. b. brucei Lab 110 EATRO, a strain susceptible to arsenical-based and diamidine trypanocides (*in vitro* and *in vivo*), and *T. b. rhodesiense* KETRI 243 clone As 10–3, a clone of a field isolate strain that is resistant to these agents, were grown, isolated from rats, and separated from blood as previously described [8]. Cells were suspended in PBSG, and were maintained in an ice bath at a concentration of 1×10^8 or 1×10^9 cells/mL, depending on the nature of the assay, as described below. All experiments were done at room temperature (approximately 25°).

2.2. Kinetics of uptake

Trypanosomes of both strains at 1×10^8 cells/mL in PBSG were incubated with various concentrations of [8-^{14}C]adenosine or [8-^{14}C]MTA (10 μM to 2.5 mM) for a period of 1 min at room temperature. Assays were carried out as described in Goldberg *et al.* [15,16] and adapted from the methods described by L'Hostis *et al.* [20] and Aronow *et al.* [21].

2.3. Effects of specific intermediates of methionine and MTA metabolism and analogs on transport

Competition of transport was carried out for both *T. b. brucei* and *T. b. rhodesiense,* as previously described in Goldberg *et al.* [16], by co-incubation with [8-^{14}C]MTA (20 μM) or [8-^{14}C]adenosine (26 μM). The concentrations of MTA or adenosine are significantly lower than their respective apparent K_m values and the concentrations of challenging intermediates or analogs. Specific intermediates of AdoMet utilization and methionine recycling were co-incubated with [8-^{14}C]MTA or [8-^{14}C]adenosine. These were: adenosine (100 μM), AdoMet (100 μM), inosine (100 μM), KTMB (100 μM), methionine (100 μM), and the MTA analog HETA (1 μM). Likewise, the polyamines spermine, spermidine, and putrescine (each at 1000 μM) were competed with both MTA and adenosine.

2.4. HPLC analysis

The overall methodology for HPLC analysis has been described previously [8,15–17,22]. Retention times (RT in minutes) for radiolabeled standards were: adenosine (22:00), adenine (20:00), ADP (4:00), ATP (6:00), and MTA (34:00).

2.5. Radiolabel and calculations

[8-^{14}C]Adenosine (59.8 mCi/mmol) and [8-^{14}C]MTA (51 mCi/mmol) were obtained from Dupont (NEN) Research Products (Boston, MA).

2.6. Calculations

All values were determined in duplicate (N = 5 determinations) and standard deviations are presented with the

mean values for Henri–Michaelis–Menten plots of transport. All graphs were plotted on Jandel Sigma Plot for Windows (Jandel Scientific, San Rafael, CA); linear Hanes–Woolf plots of Henri–Michaelis–Menten results were produced by utilizing linear regression analysis extended through the Y axis. All values expressed as kinetic constants were graphically derived from Hanes–Woolf linear plot [23].

Competition between adenosine or MTA and intermediates was analyzed as previously described in Goldberg *et al.* [16] as the difference between the slopes of controls and cells co-incubated with the competing substance. Time points for these competitions were 0.5 to 10 min. Percent inhibition is inhibition of the rate of uptake: nmol/min (in presence of agent)$^{-1}$ ÷ nmol/min (control rate) × 100 = % inhibition.

3. Results

3.1. Kinetics of uptake

The transport of MTA by *T. b. brucei* and *T. b. rhodesiense* (1×10^8 cells/mL; in PBSG) was studied by varying the MTA concentrations from 10 μM to 2.5 mM with 10 μM [8-^{14}C]MTA (Fig. 1A). The kinetic constants for transport were derived by Hanes–Woolf analyses [23] of Henri–Michaelis–Menten results (Fig. 1B). The derived values are expressed in Table 1. Transport of MTA was analyzed further by a Hill log-log plot [23] for co-operativity of transport (graphic representations are not shown). The slope (N) for both strains gave values of ~1. At this value of N = 1, $K' = [S]^n_{0.5} = K_m$ [23]. The K' values from this analysis were 0.9 and 2.0 mM for *T. b. rhodesiense* and *T. b. brucei*, respectively. These values are in agreement with those obtained by Hanes–Woolf analysis for MTA transport (Table 1). The concentration-dependent uptake of [8-^{14}C]adenosine (10 μM to 1 mM) by *T. b. brucei* and *T. b. rhodesiense* was analyzed with and without 5 mM inosine ([5]; Figs. 2 and 3) to delineate adenosine transport by the P_2 transporter (adenosine/adenine) from the combined transport by P_1 (inosine/adenosine) + P_2 transporters. Kinetic constants were derived by Hanes–Woolf analysis (Fig. 2, B–D and Fig. 3, B–D; [23]), and were compared with those obtained for MTA (Table 1). From the Hanes–Woolf analysis it can be determined that the *T. b. brucei* high-affinity adenosine transporter (in the presence of 5 mM inosine) had a K_m value of approximately 0.175 mM and a V_{max} rate of transport of 7.8 μmol/L/min (Fig. 2B; Table 1). The P_1 low-affinity transporter (without inosine) had a K_m value of 0.85 mM but a V_{max} value of 34 μmol/L/min (Fig. 2D; Table 1), five times that of the P_2 transporter. The P_2 transporter had a 5-fold greater affinity for adenosine than the P_1 transporter. Likewise, the *T. b. rhodesiense* high-affinity P_2 transporter (in the presence of 5 mM inosine: K_m 0.11) had a 6-fold greater affinity for adenosine compared with the low-affinity P_1 transporter, K_m value 0.675 mM

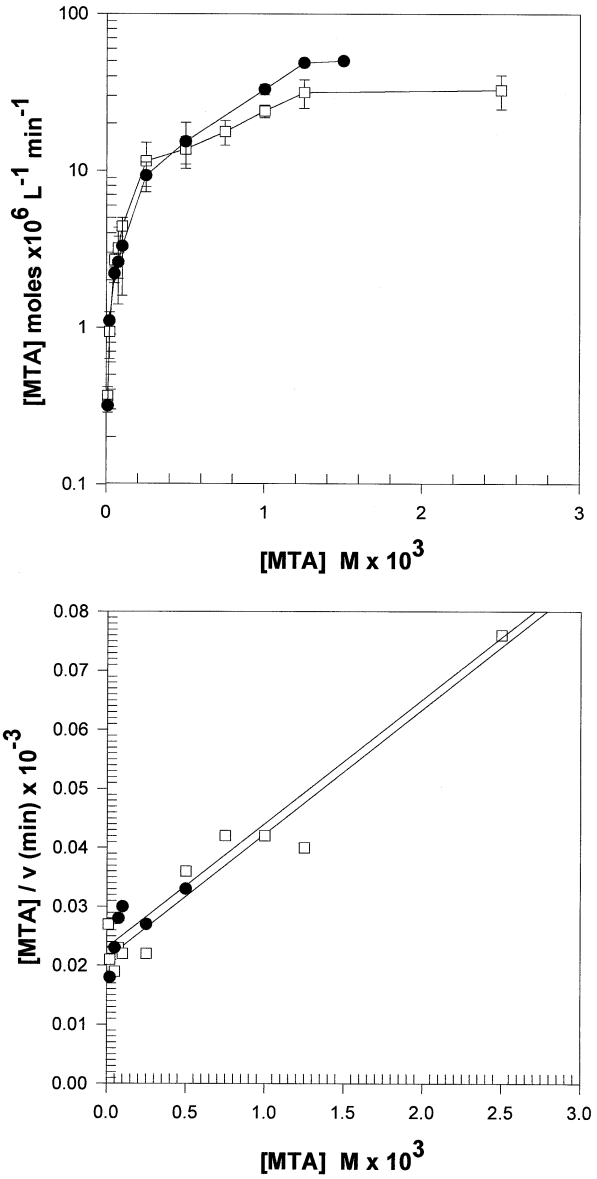

Fig. 1. MTA uptake by *T. b. brucei* and *T. b. rhodesiense* (1×10^8 cells/mL) in PBSG as described in "Materials and methods." Data were plotted as described previously. Data are expressed as means ± SD of 5 determinations. (A) Henri–Michaelis–Menten plot of uptake: (□—□) *T. b. brucei* and (●—●) *T. b. rhodesiense.* (B) Hanes–Woolf analysis [23] of Henri–Michaelis–Menten data. Values were derived by linear-regression analysis through the y-axis (Jandel Sigma Plot for Windows). Values are reported in Table 1.

(Fig. 3B, Table 1). The P_1 transporter rate, V_{max} 15.9 μmol/L/min (Fig. 2D, Table 1), was ~5 times the P_2 transport rate, V_{max} 2.9 μmol/L/min (Table 1). Comparison of both high- and low-affinity transport, P_1 and P_2, for *T. b. brucei* and *T. b. rhodesiense* showed similar patterns of difference (Table 1). The P_2 transport affinity values for these strains were comparable (0.175 vs 0.11 mM) as were the P_1 transport affinity values (0.85 vs 0.675 mM). The rates of transport by both P_1 and P_2 for *T. b. rhodesiense* were approx-

Table 1
Summation of kinetic constants for adenosine and MTA uptake

		[8-^{14}C]Adenosine						[*methyl*-^{14}C]MTC			
		$P_1 + P_2$ transporter (without inosine)[a]			P_2 transporter (with 5 mM inosine)[b]						
		K_m (mM)	V_{max} (μmol/L/min)	V_{max}/K_m[c] (min^{-1})	v_o[d] ($\times 10^{-10}$ mol/L/min)	K_m (mM)	V_{max} (μmol/L/min)	V_{max}/K_m[c] (min^{-1})	v_o[d] ($\times 10^{-10}$ mol/L/min)	K_m (mM)	V_{max} (μmol/L/min)	V_{max}/K_m[c] (min^{-1})	v_o[d] ($\times 10^{-10}$ mol/L/min)
T. b. b.	(a)[e]	0.15	6.8	0.045	4.5	0.175	7.8	0.045	4.5	1.4	70	0.05	0.5
	(b)[f]	0.85	34	0.04	4.0								
T. b. r.	(a)[e]	0.12	4.3	0.036	3.6	0.11	2.9	0.026	2.6	1.2	52.6	0.045	0.45
	(b)[f]	0.675	15.9	0.028	2.8								

Cells [*T. b. b.* (*T. b. brucei* EATRO Lab 110) and *T. b. r.* (*T. b. rhodesiense* KETRI 243 As 10-3)] at 1×10^8/mL were incubated in PBSG (pH 7.8) at room temperature with various concentrations of non-radiolabeled adenosine and MTA (10 μM to 2.5 mM) and 59.8 mCi/mmol of [8-^{14}C]adenosine or 51 mCi/mmol of [8-^{14}C]MTA. Calculations of data plots were done with Jandel Sigma Plot for Windows, utilizing Hanes-Woolf analysis of Henri–Michaelis–Menten results [23].

[a] Without inosine present in the reaction mixture.

[b] In the presence of 5 mM inosine in the incubation buffer, PBSG.

[c] V_{max}/K_m rates of "best substrate" analysis.

[d] v_0: value calculated as $k = V_{max}/K_m$ or $v_0 = k[S]$. ([S] are serum concentrations; adenosine, 1×10^{-8} M [26]; and MTA, 1×10^{-9} M [12].

[e] High affinity, P_2 transporter [5].

[f] Low affinity, P_1 transporter [5].

imately half those of *T. b. brucei* (Table 1). The P_2 transporter had a 10-fold lower affinity for MTA than adenosine. The MTA transport V_{max} rates were 9 and 18 times that of adenosine for both *T. b. brucei* and *T. b. rhodesiense*, respectively (Table 1). The MTA transport rates for both strains were comparable (Table 1).

3.2. In situ *analysis of MTA metabolism*

The *in situ* metabolism of MTA was analyzed by HPLC detection of metabolic products, as described in "Materials and methods." At 200 μM [8-^{14}C]MTA, the specific activity of MTA metabolism was $\sim$ 1.8 nmol/min/mg protein (Fig. 4) for both strains. Due to the nature of the radioisotopes available and the presence of both MTA phosphorylase and nucleosidase, both of which cleave MTA, this activity cannot be assigned to one enzyme or the other by *in situ* analysis. Only the phosphorolytic cleavage of MTA forms a product, MTR-1-P, which can be metabolized further to methionine.

3.3. Effects of intermediates of methionine metabolism on MTA and adenosine transport

T. b. brucei and *T. b. rhodesiense* at 1×10^9 cells/mL were incubated with 20 μM [8-^{14}C]MTA or 26 μM [8-^{14}C]adenosine and intermediates of methionine metabolism (100 μM) for 10 min (Table 2), and competition for MTA uptake was determined. Of the intermediates, methionine, AdoMet, and KMTB had no effect on MTA transport, and only KMTB had any significant effect on adenosine transport, reducing it by 57% for *T. b. brucei* and by 48% for *T. b. rhodesiense*. Adenosine or adenine (100 μM) each caused $\sim$80% inhibition of MTA transport in each strain. Inosine (100 μM) had no effect on MTA transport and minimally inhibited [8-^{14}C]adenosine transport (13–16% in each strain). Since MTA transport was not sensitive to inosine (100 μM), and was inhibited markedly by both adenosine and adenine, it must be transported through the P_2 (adenosine/adenine) and not the P_1 (adenosine/inosine) transporter.

3.4. Competition by polyamines for transport

The polyamines spermine and spermidine and the diamine putrescine (all at 1000 μM) were studied for competition with adenosine and MTA uptake (Table 2). None affected MTA transport. However, putrescine inhibited adenosine uptake 20%, and in the presence of saturating inosine, it inhibited adenosine transport by 68%. Likewise, spermine inhibited adenosine transport 42% in the presence of saturating inosine. Spermidine had no effect on adenosine transport (without inosine). This was not surprising since it is known that diamidines, which functionally are polyamine

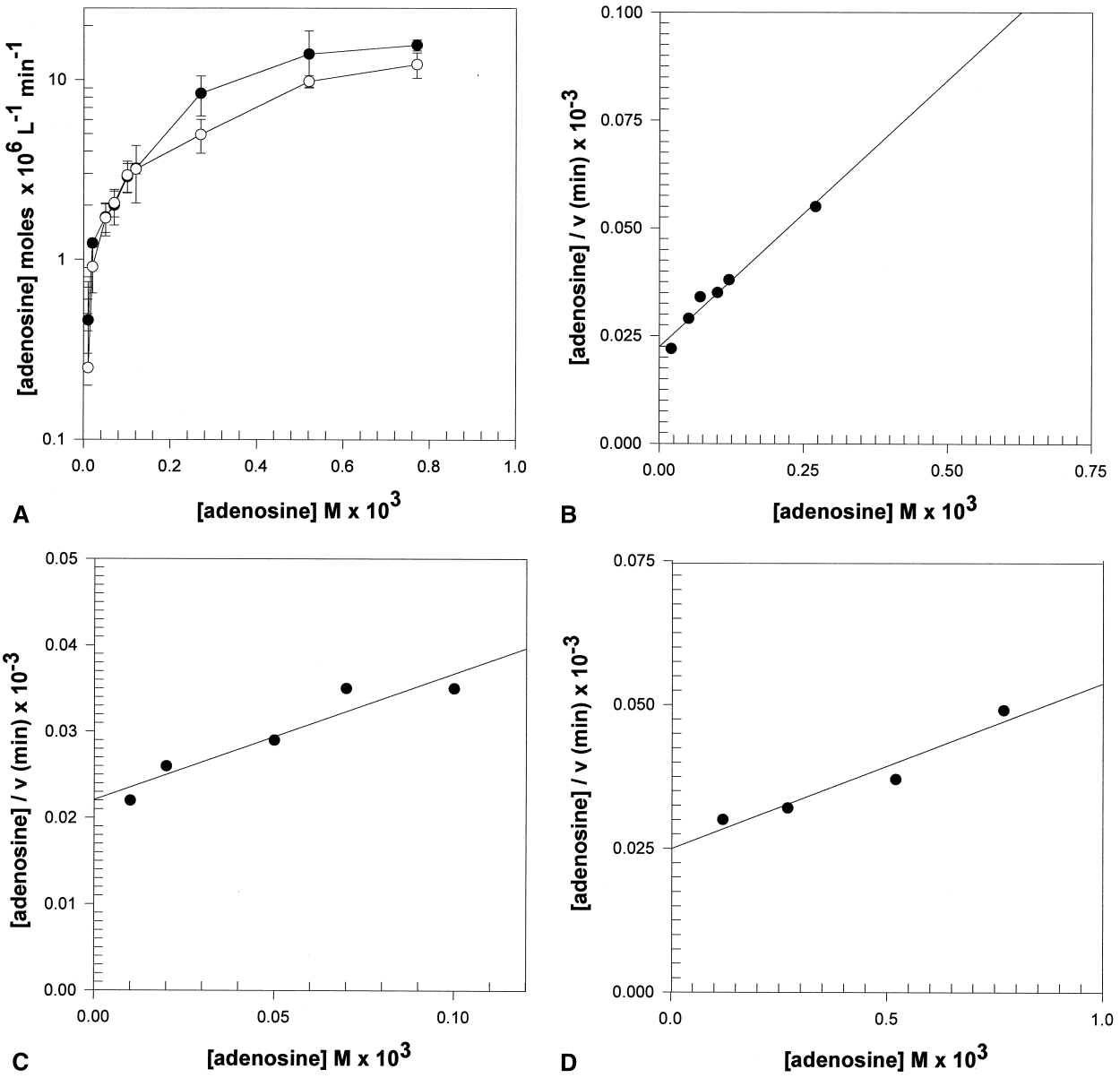

Fig. 2. *T. b. brucei* (1×10^8 cells/mL) uptake of adenosine, as described in "Materials and methods," with and without inosine. Data are expressed as means ± SD of 5 determinations. (A) Henri–Michaelis–Menten plot of uptake: (●—●) without inosine and (○—○) in the presence of 5 mM inosine. (B) Hanes–Woolf analysis [23] of Henri–Michaelis–Menten data of adenosine uptake in the presence of 5 mM inosine. Derived values are reported in Table 1. Panels C and D: Hanes–Woolf analysis of Henri–Michaelis–Menten data of adenosine uptake without inosine. (C) High-affinity adenosine transporter, P_2. (D) Low-affinity adenosine transporter, P_1. Derived values from panels C and D are reported in Table 1.

analogs, enter through the P_2 transporter [5]. Interestingly, they were not competitive with MTA.

3.5. HETA competition for transport

HETA is a substrate analog of MTA and is an interesting and successful lead compound in the treatment of experimental African trypanosomiasis [19,24,25]. It inhibited adenosine transport markedly (70–90%) in the presence of 5 mM inosine in both strains, and likewise competes for the AdoMet transporter [15,17].

4. Discussion

African trypanosomes must obtain purines from their host [2]. However, the amount of adenosine is quite low in human plasma, 1×10^{-8} to 3×10^{-7} M [26]. Other sources of adenine and adenosine present in human sera include AdoMet at $\sim 7 \times 10^{-8}$ M [27], and MTA at $\sim 1 \times 10^{-9}$ to 4×10^{-8} M [12], and these may satisfy the purine requirement. We have shown that the African trypanosomes transport AdoMet through a unique site separate from those transporting adenosine [15–17]. In the present study, it

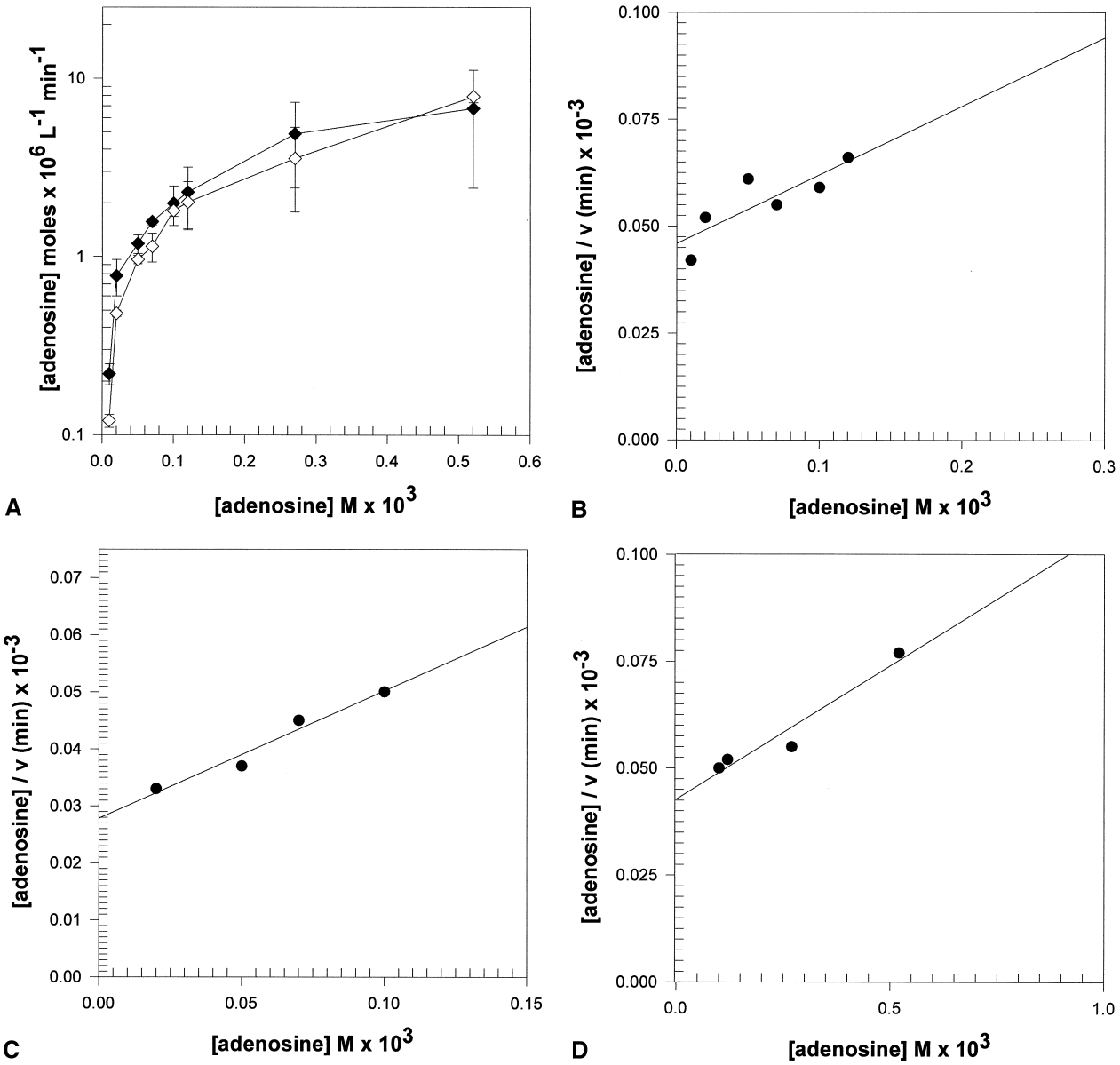

Fig. 3. *T. b. rhodesiense* (1×10^8 cells/mL) uptake of adenosine as described in "Materials and methods," with and without 5 mM inosine. Data are expressed as means ± SD of 5 determinations. (A) Henri–Michaelis–Menten plot of adenosine uptake: (◆—◆) without inosine, and (◇—◇) in the presence of 5 mM inosine. (B) Hanes–Woolf analysis [23] of Henri–Michaelis–Menten data for adenosine uptake in the presence of 5 mM inosine. Derived values are reported in Table 1. Panels C and D: Hanes–Woolf analysis of Henri–Michaelis–Menten data of adenosine uptake without inosine in the incubation medium. (C) High-affinity adenosine transporter, P_2. (D) Low-affinity adenosine transporter, P_1. Derived values from panels C and D are reported in Table 1.

appears that MTA enters through the adenosine/adenine P_2 transporter. For this reason, substrate analogs of MTA would most likely enter through this site and have the potential to significantly affect methionine and adenosine levels, as well as polyamine production.

In competitive uptake studies (Table 2) of the adenosine transporters, MTA uptake was inhibited ~80% by adenosine and adenine in both strains. This would strongly indicate that MTA was taken up through the P_2 transporter, and is reinforced by the lack of inhibition by inosine. A comparison of the kinetic constants for adenosine transport (Table 1) showed small but marked differences between drug-sensitive *T. b. brucei* and the highly melarsen oxide and pentamidine refractory *T. b. rhodesiense*. As previously described [3], the *T. b. brucei* P_2 transporter had a higher affinity for adenosine than the P_1 transporter (0.175 vs 0.85 mM), indicating that the P_1 transporter had a significantly lower affinity for adenosine (Table 1). For *T. b. rhodesiense*, the P_2 transporter likewise had greater affinity for adenosine compared with P_1 (0.11 vs 0.675 mM). Contrary to previous studies [3] in which a highly drug-resistant strain of *T. b. brucei* had lower transport activity, the *T. b. rhodesiense* resistant strain in the present study did not have a lower adenosine affinity. These findings would indicate that unlike

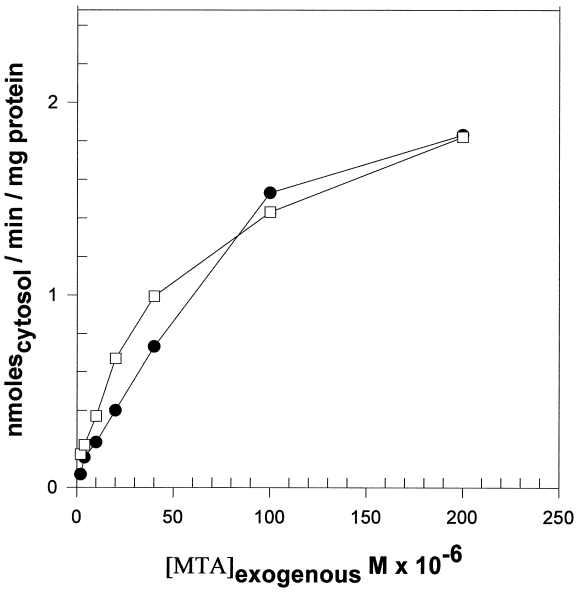

Fig. 4. HPLC analysis of MTA metabolism. The rate of MTA metabolized by MTA phosphorylase was measured as described in "Materials and methods" for 10^9 trypanosomes/mL in PBSG incubated for 1 min with various concentrations of exogenous [^{14}C]MTA (2–200 μM). Key: (●—●) *T. b. brucei*, and (□—□) *T. b. rhodesiense.* Data are calculated and plotted as described in "Materials and methods"; values are means of 2 determinations.

laboratory-derived resistant strains, drug-resistant field strains such as KETRI 243 clone As 10–3 possess a cytosolic-based mechanism(s) for resistance to pentamidine and melarsen oxide. These data also indicate that for *T. b. rhodesiense,* P_1 and P_2 transporters have similar affinity for adenosine compared with the drug-sensitive *T. b. brucei* strain (Table 1). The P_2 adenosine/adenine transporter thus remains an excellent target for new MTA analogs [28]. Both strains had very similar affinities for MTA. Likewise, their rates of transport were similar (Table 1). Although the affinities of the P_2 transporters for MTA were lower than for adenosine in both strains, the velocities with MTA were significantly greater (9- and 18-fold for *T. b. brucei* and *T. b. rhodesiense,* respectively). Taken together, these factors should result in higher levels of MTA than adenosine entering the cytosol in both strains, yielding high levels of adenosine, as well as sparing a portion of the methionine requirement. If the velocity (v_0) of MTA transport at serum blood plasma levels (~1 nM) is compared with the v_0 of adenosine transport at plasma levels (Table 1), transport of MTA should be ~ 10% that of adenosine for both strains. The v_0 rates for AdoMet at plasma levels are 2.07 and 2.88 $\times$ 10^{-9} mol/L/min for *T. b. brucei* and *T. b. rhodesiense,* respectively [14]. The v_0 rates for methionine transport are 8.9 and 7.2 x 10^{-7} mol/L/min for *T. b. brucei* and *T. b. rhodesiense,* respectively [29]. Comparison of these rates indicates that while AdoMet and MTA transport would add minimally to methionine levels, they would significantly aid these organisms in meeting their adenine/adenosine requirement. Previous studies [8,15,17] have determined that approximately 50% of AdoMet is utilized for polyamine synthesis, forming MTA as a byproduct. Utilization of MTA is a major pathway for African trypanosomes.

Table 2
Percent inhibition of adenosine and MTA uptake by nucleoside analogs or metabolites

Intermediate	Concn (μM)	% Inhibition			
		T. b. brucei		*T. b. rhodesiense*	
		MTA	Adenosine	MTA	Adenosine
Adenosine	100	81		80	
Adenine	100	81		80	
MTA	500		0, 29[a]		+[b], 48[a]
Methionine	100	0	0	0	0
AdoMet	100	0	8	0	0
KTMB	100	0	0, 57[a]	0	+, 48[a]
Inosine	100	0	16	0	13
Putrescine	1000	0	20, 68[a]	0	0, 71[a]
Spermidine	1000	0	0, 32[a]	0	0, 69[a]
Spermine	1000	0	0, 42[a]	0	0, 22[a]
HETA	1	53	30, 94[a]	40	11, 74[a]

Cells at 1 $\times$ 20^9/mL were incubated in PBSG with 20 μM [8-^{14}C]MTA or 26 μM [8-^{14}C]adenosine and specific intermediates, metabolites, or analogs for 10 min at room temperature.

[a] Inhibition in the presence of 5 mM inosine.

[b] Increase in rate.

HETA has been an exceptional lead compound in the study of the MTA salvage pathway and as a target for chemotherapy [22, 28]. MTA phosphorylase cleaves HETA to the hydroxyethylthio-1-ribose, which is then converted to hydroxyethylthioketobutyrate by a five-step pathway. The penultimate enzyme in this pathway, KMTB aminotransferase, then converts either the keto or the hydroxyethyl keto analog to methionine or hydroxyethionine.[1] Hydroxyethionine is an excellent substrate for trypanosome AdoMet synthetase,[1] which has a broad substrate specificity. As has been classically shown [30], the product, *S*-adenosylethionine, is not a substrate for transmethylases; HETA inhibits protein and lipid transmethylation significantly in the presence of exogenous [U-^{14}C]methionine [19]. HETA also competes with AdoMet for uptake [16,17] and thus may enter through at least two sites. HETA can be concentrated significantly by *T. b. brucei*: a 10 μM exogenous concentration yields a 850 μM internal concentration after a 10-min incubation [19], while it is metabolized *in situ* by trypanosome MTA-Pase at a rate equal to that of MTA. Preincubation of trypanosomes with HETA led to a 45% inhibition of [U-^{14}C]methionine incorporation into protein, when methionine was used as a methyl group source (AdoMet) in transmethylation [16,19].

MTA is an important intermediate in polyamine synthe-

[1] Bacchi CJ, Goldberg B, Metabolic conversion of HETA to hydroxyethionine through the MTA pathway of African trypanosomes, in preparation.

sis, with potential for salvage as a purine source and methionine precursor by mammalian cells. Thus, it is not surprising that perfused liver accumulates exogenous MTA at a higher rate than AdoMet and at a rate equivalent to that of methionine [13]. Similar uptake and utilization are also seen in mammalian erythrocytes, while methylthioribose is released into the medium, resulting only in adenine salvage. The process is not ATP-dependent [13]. In human lymphoblasts, cytosolic adenine is derived from the phosphorolytic cleavage of MTA through MTA-Pase [31]. MTA is a major source of adenine, and has the potential to be the major recycling source of methionine. Remethylation of homocysteine in lymphoblasts was not significant in contrast to the levels produced [32].

Other eukaryotic cells have been found to assimilate MTA, including *Entamoeba* sp. and *Candida* spp [13]. Procyclic (insect) forms of African trypanosomes grown in a medium devoid of adenine and methionine can utilize MTA as source of both [13]. In other studies, a combination of AdoMet and MTA was found to replace the B_{12} requirements of several eukaryotic microorganisms [13]. Although exogenous MTA has been reported to inhibit transmethylase systems, the high concentrations needed ($\geq$0.5 mM in most cases) appear to be unphysiological, with *S*-adenosylhomocysteine (AdoHcy) being the more specific inhibitor of these reactions. Dante *et al.* [33] have shown in transformed rat cells that cytosolic MTA, in fact, acts as a competitive inhibitor of AdoHcy hydrolase, which in turn leads to increased levels of AdoHcy. This does not appear to be the case in African trypanosomes: MTA had no effect on AdoMet uptake and accumulation or on protein methylation.[2]

Both strains of trypanosomes are capable of rapidly metabolizing pools of MTA from both transport and polyamine synthesis. There is little difference between affinities and rates of MTA transport by the P_2 transporter from these two strains with vastly different sensitivities to melarsen oxide and pentamidine. The P_2 transporter in the highly refractory strain of *T. b. rhodesiense* remains a potential port of entry for MTA analogs that could block MTA recycling and reduce the methionine pool. This was pointed out by Backlund and coworkers [10,11], who showed that cells possess a salvage mechanism for methylthio groups following polyamine synthesis. Likewise, Ogier *et al.* [34] have shown that human cells in culture can utilize KMTB (4-methylthio-2-oxobutanoate) as a substitute for methionine. KMTB is transaminated by either asparagine or glutamate in mammalian cells, and inhibition of this KMTB transaminase blocks tumor cell growth [34]. Interestingly, African trypanosomes preferentially utilize aromatic amino acids as -NH_2 donors for transamination of KMTB [7], expelling the keto-byproduct into the medium. Seed and coworkers [35–40] have shown that in mammalian hosts infected with trypanosomes, the levels of tyrosine, phenylalanine, and tryptophan are depleted in blood, while blood and urine levels of the transamination products OH-phenylpyruvate, phenylpyruvate, and indole pyruvate increase 10- to 20-fold. Thus, the flow of KMTB to methionine appears to be physiologically significant.

This study and our previous studies on metabolite transport lead us to believe that transport and metabolism of MTA phosphorylase substrate analogs provide an excellent target for new chemotherapy against this pathogen. Although the role of the trypanosome P_2 transporter in drug resistance is in question, both P_1 and the AdoMet transporter still afford an opportunity for uptake of MTA analogs, such as HETA.

[2] Goldberg B, Bacchi CJ. Effect of MTA on AdoMet and methionine transport and metabolism by African trypanosomes (2000) in preparation.

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