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Molecular and Biochemical Parasitology 74 (1995) 77–86

MOLECULAR AND BIOCHEMICAL PARASITOLOGY

Glucose transport in amastigotes and promastigotes of *Leishmania mexicana mexicana*

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Received 22 December 1994; revision received 30 August 1995; accepted 31 August 1995

Abstract

Promastigotes and amastigotes of *Leishmania mexicana mexicana* transported 2-deoxy-D-glucose (2-DOG) by a saturable process with a K_m of 24 ± 3 μM and V_{max} of 2.21 nmol min^{-1} (mg protein)$^{-1}$ for the promastigote and a K_m of 29 ± 8 μM and V_{max} of 0.13 nmol min^{-1} (mg protein)$^{-1}$ for the amastigote stage. Amastigotes incorporated 2-DOG maximally at pH 5.0, while for promastigotes the optimum was at pH 7.0. Mid-log phase promastigotes were found to accumulate 2-DOG via a stereospecific carrier-mediated process which was competitively inhibited by D-glucose and D-mannose but not L-glucose. Transport was dependent upon temperature, with a *Q10* in promastigotes of 1.83 and an optimum rate at 35°C (±4°C) with an activation energy of 50.12 kJ mol^{-1}. Stationary phase promastigotes accumulated 2-DOG at approximately twice the rate of mid-log phase promastigotes. Cytochalasin B, forskolin and phloretin were all found to inhibit human erythrocyte 2-DOG uptake but only cytochalasin B was found significantly to inhibit promastigote 2-DOG uptake. Interestingly, leishmanial 2-DOG uptake was inhibited by a series of membrane potential antagonists including the ionophore monensin, the H^+ATPase inhibitor *N,N'*-dicyclohexylcarbodiimide (DCCD) and uncoupling agent carbonylcyanide-4-(triflouromethoxy) phenylhydrazone (FCCP), as well as, the tricyclic drugs chlomipramine and imipramine, but was insensitive to the Na^+/K^+ATPase inhibitor ouabain and the antitrypanosomal drugs Pentostam and Suramin. We therefore conclude that there are significant structural and mechanistic differences between the D-glucose uptake systems of *Leishmania* and the mammalian host to merit the inclusion of glucose transporters as putative targets for rational drug design.

Keywords: Glucose transport; Amastigote; Promastigote; *Leishmania mexicana mexicana*; Homeostatis; Metabolic modification

Abbreviations: 2-DOG, 2-deoxy-D-glucose; DCCD, *N,N'*-dicyclohexylcarbodiimide; FCCP, carbonylcyanide-4-(triflouromethoxy) phenylhydrazone; PBS, phosphate-buffered saline, pH 7.3; FCS, fetal calf serum; SDM, semi-defined medium.

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SSDI 0166-6851(95)02485-V

1. Introduction

Parasites of the genus *Leishmania* cycle between mammalian and insect hosts, transforming between amastigote and promastigote life cycle stages, respectively. The physicochemical environment of the amastigote is likely to be tightly regu-

lated by homeostatic mechanisms, however, the promastigote will encounter fluctuating temperature and a chemical environment which is greatly dependent upon the nutritional status of the sandfly vector.

To survive in these two highly contrasting environments, the parasite may modify its metabolic requirements to match nutrient availability. For example, during the transformation of *Leishmania m. mexicana* from promastigotes to amastigotes there is a dramatic reduction in the catabolism of glucose between the two stages [1]. In addition, a concomitant 10-fold increase in the utilization of fatty acids was observed in the energy metabolism of the amastigote stage. Amastigotes of *L. donovani* metabolize glucose at a lower rate than promastigotes [2] but, interestingly, glucose metabolism was reported to be optimal at pH 4.0 for amastigotes while in promastigotes the optimum was pH 7.0. The pH of a *Leishmania*-infected phagolysosome is between 4.0 and 5.0 [3] while the pH of a sandfly gut is likely to be near neutral [4].

In this report we describe the characterization of D-glucose transport in amastigotes and promastigotes of *L.m. mexicana* and compare them to the mammalian erythrocyte transporter.

2. Materials and methods

2.1. Promastigote growth conditions

Promastigotes of *L. m. mexicana* (MNYC/B2/62/M379), were maintained in semi-defined medium (SDM) [5], supplemented with 10% heat-inactivated fetal calf serum (FCS) and 0.005% hemin, at a temperature of 27°C with air as the gas phase. Promastigotes were sub-passaged during the late-log phase of growth by dilution to 5×10^5 cells ml^{-1} and maintained in vitro for a maximum of 10 sub-passages to minimize adaptation to culture conditions.

2.2. Amastigote isolation

The macrophage cell line J774.G8 was maintained in RPMI 1640 growth medium, supplemented with 20 mM Hepes buffer, 2 mM L-glutamine and 20% heat-inactivated fetal calf serum, at 35°C in an atmosphere of 95% humidity, 5% CO_2. When monolayers of J774.G8 were confluent (2–4 $\times$ 10^6 ml^{-1}) they were infected with early stationary phase promastigotes at a ratio of 50 parasites per host cell. Infected monolayers were incubated for 12–24 h as above before non-phagocytosed parasites were removed by replacing growth media. After incubation for a further 24 h monolayers were washed twice with ice-cold phosphate-buffered saline (PBS), pH 7.3 (phosphate 50 mM, NaCl 150 mM) and detached using a cell-scraper (NUNC). J774.G8 cells were lysed by repeated passage through a 30 gauge needle and the amastigotes purified by Percoll density gradient centrifugation, as described by Chang [6].

2.3. Erythrocyte isolation

Human blood was collected from a single volunteer by venipuncture and erythrocytes isolated and washed in PBS by centrifugation.

2.4. Uptake assays

Cells were washed twice and resuspended in PBS (pH 7.3) containing 5 mM $MgCl_2$ to a density of 2×10^8 cells ml^{-1}. Aliquots of 100 μl of cell suspension were pre-incubated for 10 min in a shaking water bath, at 27°C for promastigotes, 35°C for amastigotes and 37°C for erythrocytes.

Assays were initiated by the addition of 100 μl of 2-deoxy-D-[1,2-^{3}H]glucose (^{3}H-2 DOG), at a specific activity of 0.45 $\mu Ci\ ml^{-1}$. Uptake was terminated by the addition of 1 ml of ice-cold PBS, containing 1% formaldehyde, and high speed microcentrifugation (4°C, 10 000 $\times$ g for 20 s). Supernatants were aspirated with a fine needle and the pellet washed with 1 ml ice cold PBS and re-centrifuged. The supernatant was aspirated and the pellet solubilized in 1 ml of 0.2 M NaOH, 0.1% Triton X-100 in PBS. Aliquots of the cell lysate were used for protein determination by the Lowry assay [7]. Radioactivity was determined in Cocktail T scintillant using a Packard Tricarb scintillation counter.

For uptake assays performed at various pHs, PBS was replaced by a basal salts solution, as described by Mukkada et al. [2].

2.5. Statistical analysis of kinetic data

K_m and V_{max} values were determined by fitting substrate saturation data to the Michaelis-Menten equation ($V = V_{max}\ S/[K_m + S]$) using the

Kaleidagraph program (Synergy Software) employing the Levenberg-Marquardt algorithm. Double reciprocal plots were constructed and fitted by least squares linear regression using the Cricket Graph III program (Computer Associates). Affinity of inhibition (K_i) was determined by plotting V/V_i (where V is V_{max} in absence of inhibitor, V_i is V_{max} in presence of inhibitor) against inhibitor concentration. The K_i was indicated by the intercept on the x-axis of a line of best fit plotted by least squares linear regression using the Cricket Graph III program (Computer Associates).

2.6. Materials

2-Deoxy-D-[1,2-^{3}H]glucose (40 Ci mmol^{-1}) was purchased from Amersham Life Sciences, UK; fetal calf serum was purchased from Gibco BRL, UK, and heat inactivated at 56°C for 30 min; Percoll was purchased from Pharmacia UK; Cocktail T was purchased from BDH, UK; all inhibitors were purchased from Sigma Chemicals, UK, and all other chemicals used were of analytical grade. The tricyclic drugs chlomipramine and imipramine were generous gifts from Ciba Geigy, UK, and Suramin and Pentostam were supplied by Bayer Pharmaceuticals, UK, and Wellcome Foundation plc, UK, respectively.

3. Results

When 2-DOG transport in *L. m. mexicana* mid-log phase promastigotes was determined as a function of time (Fig. 1) and accumulation was found to be linear, at approximately 1.6 nmol min^{-1} (mg protein)$^{-1}$, for at least 10 min. After 15 min the rate of uptake was greatly reduced and the cellular levels of 2-DOG reached a plateau between 25 and 30 nmol (mg cell protein)$^{-1}$.

Several uptake assay protocols [8–10] were tested in comparison to the formaldehyde procedure developed in these studies. The vacuum filtration [8,9] and centrifugation through oil protocols [10] give the most reproducible results but are labour intensive by comparison to the formaldehyde procedure. Interestingly, all three protocols tested give almost identical results to those reported in Fig. 1.

Cellular accumulation of 2-DOG was

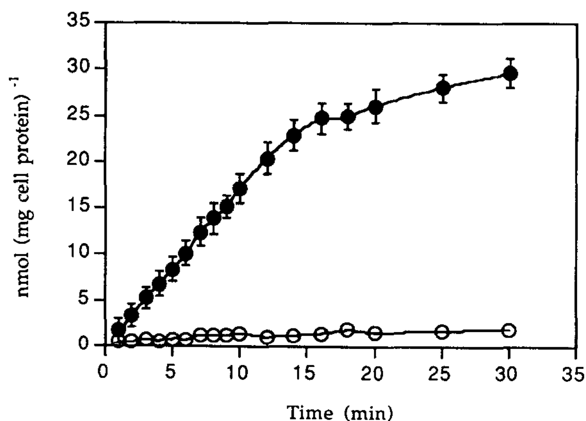

Fig. 1. Transport of 2-DOG by mid-log phase promastigotes of *L. m. mexicana*. Assayed as described in Materials and methods (●) and after pretreatment with formaldehyde 1.0% (○).

dramatically inhibited in cells which were preincubated for 10 min with 1% formaldehyde (Fig. 1). Indeed, recent studies on inositol and fatty acid transport in *Leishmania* support this suggestion (Lux and Hart, unpublished data).

Efflux of ^{3}H-2-DOG from leishmanial promastigotes is rapid when the medium is supplemented with 10 mM 2-DOG or 10 mM D-glucose (Fig. 2). More than 50% of the 2-DOG associated with the cell was released within 5 min.

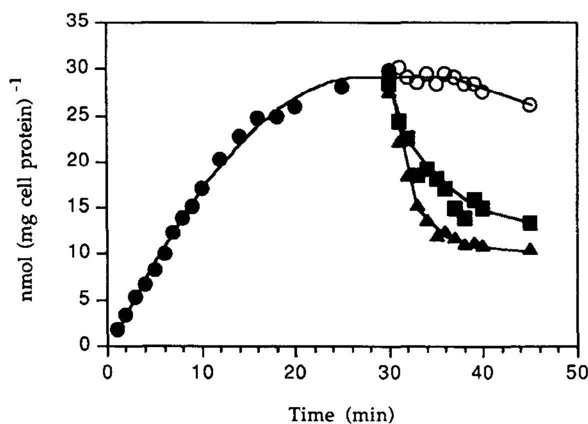

Fig. 2. Efflux of intracellular ^{3}H-2-DOG with 2-DOG, D-glucose and L-glucose by promastigotes of *L. m. mexicana*.. After 30 min incubation in 0.1 mM ^{3}H-2-DOG, unlabelled sugars were added: 10 mM L-glucose (□); 10 mM D-glucose (■); 10 mM 2-DOG (▲).

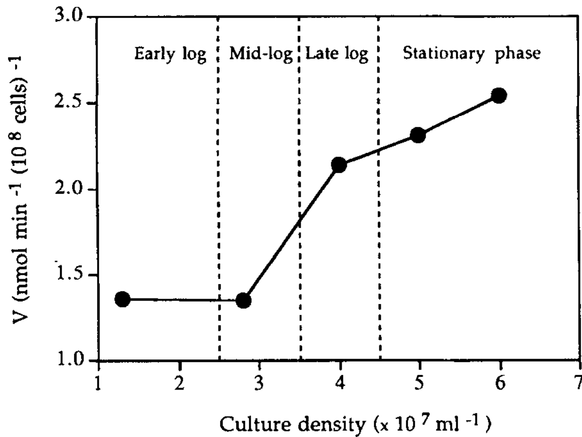

Fig. 3. Transport of 2-DOG by promastigotes of *L. m. mexicana* isolated from cultures at various stages of growth from early log to stationary phase. Velocity of 2-deoxy-D-glucose transport was determined during a 10 min incubation in 0.1 mM 2-DOG at 27°C.

However, even after 15 min more than 30% was found to be intracellular and therefore presumed to be the sequestered pool of phosphorylated 2-DOG. In comparison, when promastigotes were incubated with 10 mM L-glucose, efflux of 2-DOG was less than 10%, even after 15 min, demonstrating the stereo-specificity of the transporter.

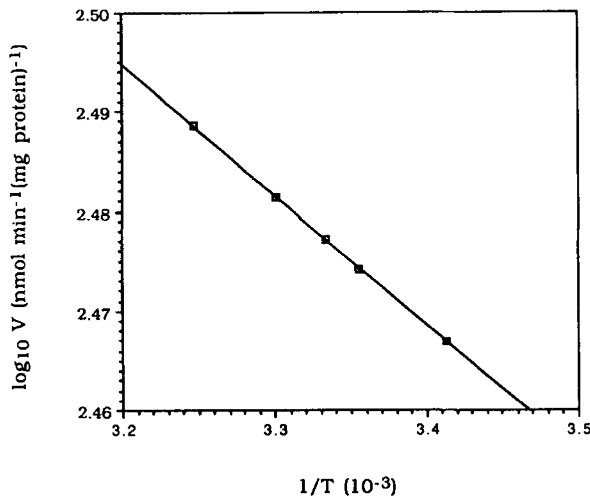

Fig. 4. Arrhenius plot of the transport of 2-DOG by mid-log phase promastigotes of *L. m. mexicana*.

The rate of D-glucose accumulation during promastigote growth was determined (Fig. 3). Promastigotes in both early and mid-log phases of growth accumulated 2-DOG at a rate of 1.1 nmol min^{-1} (mg protein)$^{-1}$. This rate increased to 1.6 during late-log phase promastigotes and to as high as 1.9 in stationary phase cells which were routinely composed of approximately 45% metacyclic promastigotes, 25% late log-phase promastigote and 30% rounded-up stationary phase promastigotes, as judged by morphology (data not shown).

It is noteworthy that leishmanial cell protein content falls during growth from log phase to stationary phase (data not shown). Therefore the rate

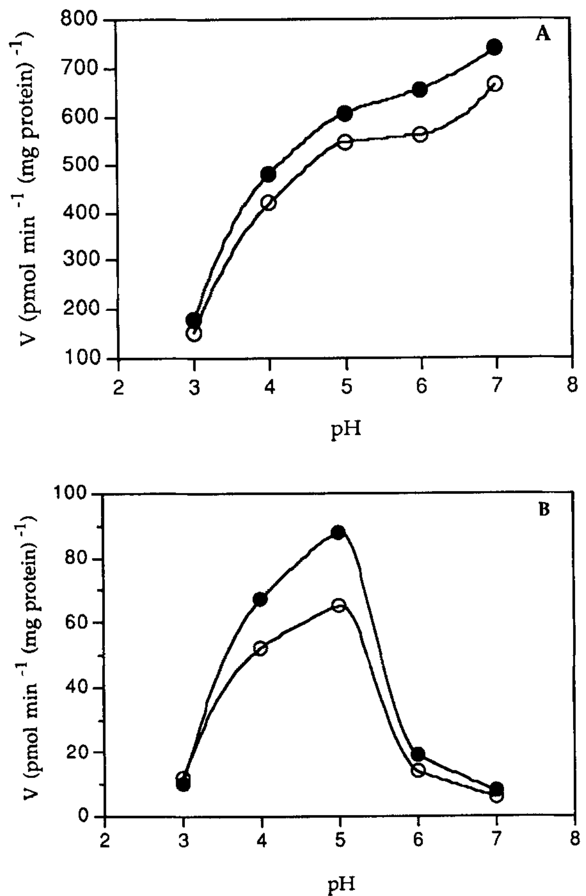

Fig. 5. Effect of pH upon transport of 2-DOG by mid-log phase promastigotes (A) and freshly-isolated amastigotes (B) at 35°C (●) and at 27°C (○).

of glucose uptake in metacyclic promastigotes is more than 2-fold higher than in early and mid-log phase promastigotes. We suggest therefore that an elevated transport and utilisation of glucose may be important in metacyclogenesis.

The D-glucose uptake system of the promastigote was further characterized by investigating the effect of temperature upon the rate of 2-DOG uptake. Experiments were performed at 4°C and at a range of temperatures between 20 and

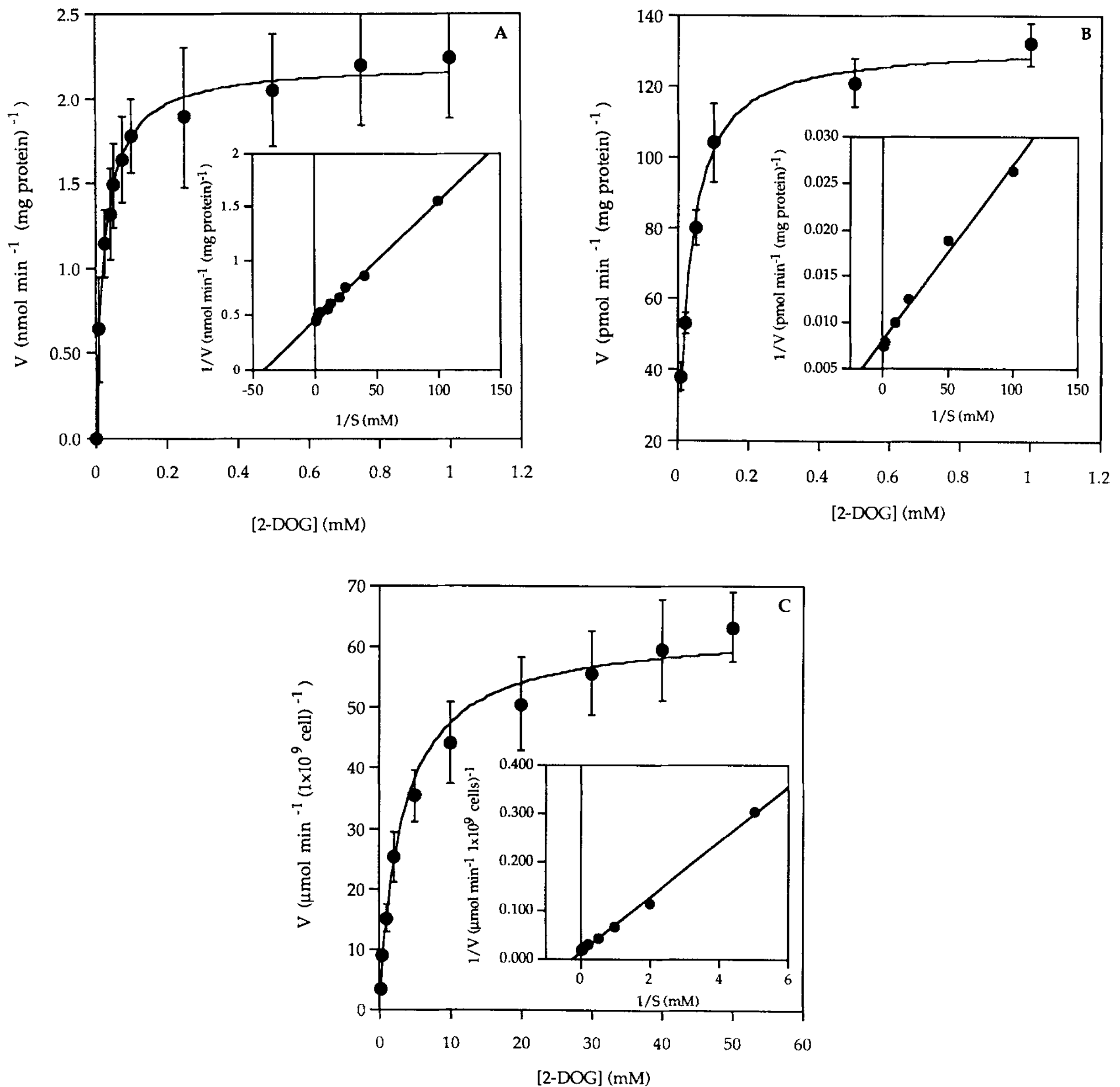

Fig. 6. Substrate saturation curves for 2-DOG measured in mid-log phase promastigotes at 27°C, pH 7.0 (A), in freshly isolated amastigotes at 35°C, pH 5.0 (B), and in freshly isolated human erythrocytes at 37°C, pH 7.0 (C). The respective kinetic transformations (as described in Materials and methods) are shown as inserts.

40°C and data are presented as an Arrhenius plot (Fig. 4).

The rate of 2-DOG accumulation in promastigotes was reduced 40-fold at 4°C compared with cells studied at the normal in vitro culture temperature of 27°C. At 40°C a dramatic decrease in the rate of uptake of approximately 30-fold was observed when compared to the uptake rates at 35°C. These data was used to determine the temperature quotient, Q_{10}, and the energy of activation, E_a, which were 1.83 and 50.12 kJ mol^{-1}, respectively.

Studies of the initial rate of 2-DOG uptake as a function of environmental pH showed that uptake in promastigotes (Fig. 5A) was most rapid at pH 7.0 and somewhat reduced at pH 5.0–6.0 and dramatically decreased at pH 4.0 and below. Conversely, uptake in amastigotes was optimal at pH 5.0, some 10-fold higher than at pH 6.0–7.0, and only marginally reduced at pH 4.0 (Fig. 5B). Amastigotes thus accumulated glucose optimally at a low environmental pH of between 4.0 and 5.0, while promastigotes exhibited optimal glucose uptake at pH 7.0. However, it should be noted that optimal rates of glucose uptake were 10-fold higher in promastigotes when compared to amastigotes.

Initial uptake rates of D-glucose were determined at a range of external 2-DOG concentrations in both amastigotes and promastigotes of *L. m. mexicana* and in human erythrocytes. In all cases the relationship between rate and substrate concentration appeared to follow Michaelis-Menten kinetics (Fig. 6).

There was no significant difference in the mean affinity constant (K_m) for 2-DOG between amastigotes (29 ± 8 μM) and promastigotes (24 ± 3 μM) (Table 1). These affinity constants were determined at pH 5.0 and 7.0 for amastigotes and promastigotes respectively. For promastigotes, there was no significant change in affinity when assays were performed at pH 5.0 (data not shown), while for amastigotes the low rate of 2-DOG uptake at pH 7.0 makes it impossible to construct a substrate saturation curve.

The V_{max} of amastigote glucose uptake (0.132 nmol min^{-1} (mg protein)$^{-1}$) was some 20-fold lower than that of promastigotes (2.21 nmol min^{-1} (mg protein)$^{-1}$). Since promastigotes can contain 5–10-fold more protein per cell than amastigotes, the difference in V_{max} is sharply accentuated when promastigote and amastigote are compared per cell. In contrast, erythrocytes showed a much lower affinity (3.22 mM) and a very much higher V_{max} (62.9 μmol min^{-1} $(1 \times 10^9$ cells)$^{-1}$).

Transport of 2-DOG in the presence of various glucose analogues indicates that uptake is competitively inhibited by both D-glucose and D-mannose with apparent K_i values of 32.1 and 60.7 μM, respectively (Table 2). In contrast, L-glucose does not show any inhibition, demonstrating the stereo-specificity of the transporter.

The effect of a number of known glucose uptake inhibitors was tested and the initial rates of 2-DOG uptake determined after pre-incubation for 10 min with the putative inhibitors. Where inhibitors were solubilized in organic solvents (dimethyl sulphoxide or ethanol) controls were performed to investigate the effect each solvent on glucose uptake. In no case did the final concentration of solvent exceed 0.5% and no significant inhibition of glucose uptake was observed at these final concentrations (data not shown).

Very interesting differences were observed in the sensitivity of the leishmanial and mammalian D-

Table 1
Kinetics of glucose transport in *L.m. mexicana* and human erythrocyte

	K_m [a]	V_{max}
L.m. mexicana promastigotes	24 (± 3) μM	2.21 nmol min^{-1} (mg protein)$^{-1}$
L.m. mexicana amastigotes	29 (± 8) μM	0.13 nmol min^{-1} (mg protein)$^{-1}$
Human erythrocyte	3.22 (± 0.3) mM	62.9 μmol min^{-1} $(1 \times 10^9$ cells)$^{-1}$

[a]Mean from three to five determinations ± SE.

Table 2
Comparison of potential inhibitors of 2-DOG transport in promastigotes of *L. m. mexicana*

Inhibitor	Category	K_i (μM)	IC_{50} (μM)
D-Glucose	Hexose sugar	32.1	n.d.
L-Glucose	Hexose sugar	> 10 000	n.d.
D-Mannose	Hexose sugar	60.7	n.d.
Cytochalasin B	Fungal metabolite	n.d.	48.4
Cytochalasin E	Fungal metabolite	n.d.	> 10 000
Forskolin	Plant diterpene	n.d.	5,800
Phloretin	Plant glucoside	n.d.	1,300
Ouabain Na^+/K^+	ATPase inhibitor	n.d.	> 10 000
Monensin	Ionophore	n.d.	76
DCCD H_+	ATPase inhibitor	n.d.	21
FCCP	Proton ionophore	n.d.	1.4
Chlomipramine	Tricyclic antidepressant	n.d.	43
Imipramine	Tricyclic antidepressant	n.d.	85
Suramin	Antiparasitic drug	n.d.	> 10 000
Pentostam	Antiparasitic drug	n.d.	> 10 000

n.d; not determined

glucose uptake systems to known inhibitors (Table 2). Cytochalasin B, forskolin and phloretin are all potent inhibitors of the erythrocyte D-glucose transporter [12–15]. In sharp contrast only cytochalasin B produced any significant inhibition of the leishmanial counterpart, with an IC_{50} of 48.4 μM which is some 10-fold higher than that typically reported for mammalian transporters.

Studies on *L. donovani* have suggested a role for proton motive force in glucose uptake [8,16]. The effect of membrane potential inhibitors upon D-glucose uptake in leishmanial promastigotes was investigated to elucidate possible links between membrane energy transduction and the uptake process in *L. m. mexicana*. 2-DOG uptake was insensitive to the Na^+/K^+-ATPase inhibitor ouabain, while the IC_{50} of the ionophore monensin was 76 μM, of the proton pump inhibitor DCCD 21 μM, and of the uncoupler FCCP 1.4 μM. Most significantly two tricyclic antidepressant drugs, Chlomipramine and Imipramine, which have been shown to possess antileishmanial activity [17,18], were found to inhibit 2-DOG uptake at the comparatively low concentrations of 43 and 85 μM, respectively.

This level of sensitivity is highly comparable to the effective antileishmanial concentrations in vitro (Mayer and Hart, unpublished data) and may suggest that D-glucose uptake is, at least in part, involved in the mechanism of action of these drugs which are known to disrupt membrane potentials in other systems. In contrast, the antiparasitic drugs Suramin and Pentostam which are suggested to perturb glycolysis in *Trypanosoma* and *Leishmania*, respectively, were found to have no significant effect upon 2-DOG uptake even at the highest concentration used (10 mM).

4. Discussion

2-DOG uptake in *Leishmania* has been studied in the promastigote stages of *L. tropica* and *L. donovani* [8,10,16,19,20] and the kinetic data presented here is in good agreement with these reports. It is important to study glucose uptake activity in isolation from glycolysis and this can be achieved by the use of non-metabolizable D-glucose analogues.

Interestingly, 2-DOG is seemingly rather actively phosphorylated by leishmanial hexokinase (Ref. [10], and Hart and Hammond, unpublished data). Hexokinase in *Leishmania* is uniquely located in glycosomal microbodies and our preliminary data suggest that phosphorylated 2-DOG may be extensively sequestered in this subcellular compartment.

Leishmanial transporter studies [8,16] have

shown that D-glucose uptake is competitively inhibited by D-glucose analogues which are substituted in the C-2 position, such as 2-DOG, D-fructose and D-mannose. Our results confirm this finding, and the low K_i value (32.1 μM) for the inhibition of promastigote 2-DOG uptake by D-glucose supports the use of 2-DOG as a reporter for D-glucose uptake.

In these studies we observed that the rate of D-glucose uptake in promastigotes of *L. m. mexicana* approximately doubled in the late-log and stationary phases of growth. Mukkada et al. [20] have shown that glucose utilization in *L. major* increased as cultured promastigotes approached late-log phase and persisted at some 3 times the early log phase rate in the stationary phase. There are a number of reports that stationary phase cultures of *Leishmania* promastigotes are enriched in a morphologically distinct metacyclic stage (e.g., Ref [21]). Metacyclic promastigotes are smaller non-dividing and highly motile stages which have been shown to be highly infective.

The results of Mallinson and Coombs [21] confirms our observation that approximately 40% of typical stationary cultures of *L. m. mexicana* have metacyclic-like morphology. We thus speculate that the onset of metacyclogenesis may be accompanied by an increase in glucose transport and utilisation. Interestingly Pro-1, a gene which encodes a leishmanial glucose transporter (ref. [22], and Burchmore and Landfear, unpublished data), is expressed most strongly in promastigotes of *L. m. mexicana* cultured under conditions which may induce metacyclogenesis [23].

The expression of Pro-1 is dramatically down-regulated in amastigotes of *L. enriettii* [24] and *L. m. mexicana* (Burchmore and Landfear, unpublished data). Given that there is approximately 10-fold lower activity of glycolytic enzymes in amastigotes of *L. m. mexicana*, it is not surprising that the rate of glucose uptake is some 10-fold lower than in promastigotes. To our knowledge, this is the first report of glucose uptake in the intracellular stage of *Leishmania*.

More intriguing is the observation that amastigotes accumulate glucose optimally at pH 5.0, which is close to the pH of the phagolysosome [3], while promastigotes seem to have a wider pH optima, peaking near neutrality. This supports the findings of Mukkada et al. [2] that amastigote glucose catabolism has an acidic pH optima. However, Zilberstein and Gepstein [25] have reported that *L. donovani* promastigotes, cultured at pH 4.5, show pH-independent glucose uptake activity. These seemingly variant observations might be explained by species differences.

The apparent K_m values of the promastigote and amastigote glucose uptake systems were very similar (24 and 29 μM, respectively) and unusually low for a eukaryotic cell. The value obtained here for *L. m. mexicana* is almost identical to that reported for glucose uptake in *L. donovani* promastigotes [8] and somewhat lower (by approximately 4-fold) than reported for *L. donovani* [26] and *L. tropica* [10]. Whether these differences are explained by variation in species or technique remains to be determined. Taken together, however, the reported K_m values for *Leishmania* spp. are significantly lower than those reported for mammalian glucose uptake systems.

The similar K_m values for glucose transport in promastigote and amastigote may suggest that the same transporter is expressed in both developmental stages and that due to differences in the membrane environments of the respective transporters they demonstrate varient pH optima. It is, however, equally possible that two separate transporters with a similar K_m are operative which would also explain the respective differences in pH optima.

The sensitivity of leishmanial glucose uptake to inhibition by various potential inhibitors reveals further aspects in which the parasite glucose uptake system differs from that of its mammalian host. Phloretin [12], cytochalasin B [13] and forskolin [14] are all well characterized inhibitors of human erythrocyte glucose transport and have been shown to inhibit glucose uptake in a wide range of other cell types. Their effective inhibition of erythrocyte glucose uptake was confirmed in our studies, however, in contrast, the leishmanial glucose transporter demonstrated only moderate sensitivity to cytochalasin B and none at all to phloretin and forskolin.

Our observations that *L. m. mexicana* glucose uptake is sensitive to inhibition by the ionophore

monensin, the H^+ATPase-inhibitor DCCD and to the uncoupling agent FCCP may be added to considerable biochemical evidence suggesting that glucose uptake in *L. donovani* is an active process, coupled to transmembrane proton flux [8,16,27]. Such a symport system has not been identified in any mammalian system. However some doubt has recently been raised, as kinetic data may be re-interpreted to suggest facilitative glucose uptake [28,29].

Genes with homology to both facilitative and active glucose transporter genes have been cloned from *Leishmania* (reviewed in Ref. [30]) and it is possible that both modes of transport operate, as appears to be the case for proline transport in *L. donovani* (Dan Zilberstein, personal communication). The functional co-existence of both active and facilitative transporters in the same membrane cannot be reconciled unless their expression is developmentally regulated or their activity is modulated by environmental conditions such as pH or substrate availability. Existing data may also be explained by postulating the existence of a facilitative glucose transporter in the plasma membrane and an active transporter in the membrane of the glycosome [28].

By definition, active uptake involves accumulation against a concentration gradient. Assuming that the cell volume and protein content of *L. m. mexicana* is similar to that measured for *L. donovani* [8], an uptake of 30 nmol (mg cell protein)$^{-1}$ implies an intracellular 2-DOG concentration of approximately 7 mM. This represents uptake against a concentration gradient, unless more than 95% of intracellular 2-DOG has been metabolically converted or sequestered. In contrast our efflux studies suggest that only some 30% of intracellular 2-DOG may be phosphorylated and sequestered.

Tricyclic antidepressants possess antileishmanial activity and also reduce transmembrane proton motive force in *Leishmania* and perturb uptake functions [17,18]. Our observation that tricyclic drugs are effective inhibitors of *L. m. mexicana* glucose uptake is further indirect evidence of a role for proton motive force in leishmanial glucose uptake and suggests a putative mechanism of antileishmanial action for tricyclic compounds.

In summary, our results suggest that the D-glucose uptake system of *L. m. mexicana* is at significant variance to that of the mammalian host. Differences were found in substrate affinity as well as in sensitivity to a series of competitive inhibitors of glucose uptake and membrane potential antagonists.

Acknowledgements

We thank Julia Eckersley for excellent technical assistance. Dr Scott Landfear and Professor Fred Opperdoes are also thanked for helpful comments during the preparation of the manuscript. RJSB held a SERC postgraduate studentship.

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