SHORT COMMUNICATION

Further Evidence for the Existence of a Membrane Potential in *Trypanosoma brucei brucei*

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The distribution of $^{137}\text{Cs}^+$, in the presence of valinomycin, has been used to measure the magnitude of the membrane potential ($\Delta\psi$) in bloodstream forms of *Trypanosoma brucei brucei*. Values of the $\Delta\psi$ falling in the range -100 mV to -160 mV were observed and the maintenance of this $\Delta\psi$ was sensitive to certain ionophores and protonophores.

INTRODUCTION

Despite recent investigations no definitive mechanism for energy-coupling to active transport has been proposed to operate in trypanosomes or trypanosomatids (Voorheis, 1980; Midgley, 1978). A membrane potential ($\Delta\psi$) plays a key role as a driving force for the uptake of a wide range of nutrients in both prokaryotic and eukaryotic micro-organisms (Hamilton, 1975; Eddy, 1978) and evidence has recently been presented for the existence of such a gradient across the cytoplasmic membrane of *Trypanosoma brucei brucei* (Midgley, 1983). The evidence was based on the interaction of the fluorescent probe 2-(dimethylaminostyryl)-1-ethylpyridinium with washed cell suspensions of the organism. Since this was essentially an empirical approach further evidence for the existence of a $\Delta\psi$ has been sought using more direct techniques.

METHODS

Growth and harvesting of the organism. The organism used was *Trypanosoma brucei brucei* 8/18 maintained, grown for experimental work in laboratory rats, and harvested as previously described (Midgley, 1983). The buffer solution used for resuspension of the organism is referred to as Na-HEPPS-glucose and had the following composition: 173 mM-4-(2-hydroxyethyl)-1-piperazine-propane sulphate (HEPPS) adjusted to pH 8.0 with NaOH; 50 mM-glucose.

Measurement of $\Delta\psi$. This was based on the measurement of accumulation ratios for $^{137}\text{Cs}^+$, in the presence of valinomycin, or for labelled methyltriphenylphosphonium ion using a rapid filtration technique and principles previously described (Horan *et al.*, 1978; Sissons & Midgley, 1981). The composition of the incubation medium was as follows: organism (~ 0.3 mg protein ml^{-1}) in Na-HEPPS-glucose, 4.45 ml; radiochemical, either 0.05 ml $^{137}\text{CsCl}$ (2.25 mM, 5 $\mu\text{Ci } \mu\text{mol}^{-1}$) or 0.045 ml [^3H]methyltriphenylphosphonium bromide (7.2 μM , 2.8 $\mu\text{Ci } \mu\text{mol}^{-1}$) or 0.045 ml [^{14}C]methyltriphenylphosphonium bromide (1.1 mM, 9 $\mu\text{Ci } \mu\text{mol}^{-1}$) [1 $\mu\text{Ci} = 37$ kBq]; valinomycin, 4.5 μl of a 1 mg ml^{-1} methanolic solution, if added.

Inhibitors and ionophores were added as previously described (Midgley, 1983). All incubations were carried out at 37 °C in magnetically-stirred cylindrical vessels. Samples (0.2 to 0.6 ml) were removed as required and filtered as previously described (Midgley, 1978) using ice-cold phosphate-buffered-saline/glucose medium (Lumsden *et al.*, 1973) as the filter wash. A correction for extracellular and filter binding was derived from controls in which cells and probe were mixed on ice and rapidly sampled.

Chemicals. These were obtained from sources previously specified (Midgley, 1983). Radiochemicals were obtained from Amersham.

Fig. 1. Valinomycin-dependent uptake of $^{137}\text{Cs}^+$ by *T. b. brucei*. The experimental conditions were as described in Methods with the following variables: ○, 25 μM -CsCl, no valinomycin; △, 25 μM -CsCl, 1 μg valinomycin ml^{-1} ; □, 25 μM -CsCl, 0.5 μg valinomycin ml^{-1} ; ●, 50 μM -CsCl, 1 μg valinomycin ml^{-1} .

RESULTS

When washed cell suspensions of *T. b. brucei* 8/18 were incubated with $^{137}\text{CsCl}$ in the presence of glucose an uptake of Cs^+ , stimulated by valinomycin, was observed (Fig. 1). The amount accumulated was similar when valinomycin was present at 0.5 or 1.0 $\mu\text{g ml}^{-1}$. Using a value for the cell water content derived from published data (Voorheis, 1971) and the data presented in Fig. 1 allows calculation of the $\Delta\psi$, after 30 min incubation, as -154 and -157 mV at $[\text{CsCl}]$ of 50 μM or 25 μM respectively. As higher $[\text{CsCl}]$ produced depolarization, measurements were restricted to this concentration range. The value of $\Delta\psi$ measured was independent of the cell density over the range 0.2 to 0.4 $\text{mg protein ml}^{-1}$. The exact value of the $\Delta\psi$ varied, the values falling in the range -100 to -160 mV with 15 independent batches of the organism. The mean value was $-129 \text{ mV} \pm 4 \text{ mV}$ (S.E.M.).

In order to confirm that a metabolically-generated $\Delta\psi$ was being measured rather than a K^+ -diffusion potential produced on the addition of valinomycin, experiments were performed using salicylhydroxamate (SHAM). This compound inhibits respiration, due to aerobic glycolysis in this organism, and diminishes the yield of ATP from glucose (Gutteridge & Coombs, 1977). The addition of 2 mM-SHAM produced a marked inhibition of valinomycin-dependent Cs^+ accumulation: the control suspension accumulated 5.6 $\mu\text{g ion (g protein)}^{-1}$ after 20 min incubation whereas in the presence of SHAM 1.6 $\mu\text{g ion (g protein)}^{-1}$ was accumulated, corresponding to $\Delta\psi$ values of -125 mV and -90 mV respectively. In a second experiment the values were as follows: control, 4.9 $\mu\text{g ion (g protein)}^{-1}$ ($\Delta\psi = -121$ mV); plus SHAM, 0.4 $\mu\text{g ion (g protein)}^{-1}$ ($\Delta\psi = -56$ mV).

The valinomycin-dependent uptake of Cs^+ was completely abolished by the addition of 20 μM -carbonyl cyanide *m*-chlorophenylhydrazine, 2 μM -tetrachlorosalicylanilide, gramicidin (1 $\mu\text{g ml}^{-1}$), 5 mM-KCl or 100 μM -tetraphenylphosphonium chloride. These observations indicate that the $\Delta\psi$ measured using the Cs^+ -valinomycin technique has a similar sensitivity to the agents that effectively prevented the increase in fluorescence intensity observed on adding 2-(dimethylaminostyryl)-1-ethylpyridinium to suspensions of *T. b. brucei* (Midgley, 1983).

Further evidence for the existence of a $\Delta\psi$ was obtained using the lipophilic methyltriphenylphosphonium cation. When washed cell suspensions were incubated with low concentrations (7.2 nM to 11 μM) of this probe an energy-dependent uptake that reached a plateau within 20 to 30 min was observed. This uptake was sensitive to the compounds that inhibited valinomycin-dependent Cs^+ uptake, including the combination of 5 mM-KCl and valinomycin (1.0 $\mu\text{g ml}^{-1}$).

When the two techniques were quantitatively compared using the same batch of cells under identical incubation conditions, the value of the $\Delta\psi$ computed from the data obtained with methyltriphenylphosphonium ion (7.2 nM) was significantly different from that computed from Cs^+ distribution; in two experiments the $\Delta\psi$ values were -144 vs -119 mV and -157 vs -104 mV, the results obtained with the lipophilic cation indicating a greater degree of membrane polarization.

DISCUSSION

The results presented here confirm the existence of a significant $\Delta\psi$ across the cytoplasmic membrane of bloodstream forms of *T. b. brucei*. The reason for the disagreement between the values calculated using the data from Cs^+ -valinomycin experiments and those obtained using methyltriphenylphosphonium ion is currently unknown but there have been a number of reports indicating limitations to the use of this probe in other systems (Barts *et al.*, 1980; Lolkema *et al.*, 1982). Values obtained using the valinomycin system are usually considered to reflect the true value more closely as significant intracellular binding of inorganic cation is considered not to occur, in contrast to the behaviour of the lipophilic cations (Rink, 1982). The calculation of the $\Delta\psi$ value assumes uniform distribution of the probe throughout the cell water. As this is unlikely in a eukaryotic organism even the values obtained using Cs^+ -valinomycin must be considered approximate.

Major remaining questions are the roles of the $\Delta\psi$ detected in this study and how this gradient is generated. A partial answer to the first question is available as it has been demonstrated that when the cell membrane was depolarized by the addition of suitable ionophores then the active transport of amino acids was also inhibited (M. Midgley, unpublished observations).

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