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Thiamin Transport by Human Erythrocytes and Ghosts

D. Casirola, C. Patrini, G. Ferrari, and G. Rindi
Institute of Human Physiology, University of Pavia, 27100 Pavia, Italy

Summary. Thiamin transport in human erythrocytes and resealed pink ghosts was evaluated by incubating both preparations at 37 or 20°C in the presence of [^{3}H]-thiamin of high specific activity. The rate of uptake was consistently higher in erythrocytes than in ghosts. In both preparations, the time course of uptake was independent from the presence of Na^+ and did not reach equilibrium after 60 min incubation. At concentrations below 0.5 μM and at 37°C, thiamin was taken up predominantly by a saturable mechanism in both erythrocytes and ghosts. Apparent kinetic constants were: for erythrocytes, K_m = 0.12, 0.11 and 0.10 μM and J_{max} = 0.01, 0.02 and 0.03 pmol · μl^{-1} intracellular water after 3, 15, and 30 min incubation times, respectively; for ghosts, K_m = 0.16 and 0.51 μM and J_{max} = 0.01 and 0.04 pmol · μl^{-1} intracellular water after 15 and 30 min incubation times, respectively. At 20°C, the saturable component disappeared in both preparations. Erythrocyte thiamin transport was not influenced by the presence of D-glucose or metabolic inhibitors. In both preparations, thiamin transport was inhibited competitively by unlabeled thiamin, pyrithiamin, amprolium and, to a lesser extent, oxythiamin, the inhibiting effect being always more marked in erythrocytes than in ghosts. Only approximately 20% of the thiamin taken up by erythrocytes was protein- (probably membrane-) bound. A similar proportion was esterified to thiamin pyrophosphate. Separate experiments using valinomycin and SCN^- showed that the transport of thiamin, which is a cation at pH 7.4, is unaffected by changes in membrane potential in both preparations.

Key Words thiamin transport · human erythrocytes · ghosts

Introduction

It is generally accepted that the transport of thiamin at low concentrations (<0.5 μM) by rat erythrocytes is a carrier-mediated process (Komai & Shindo, 1974*a*; Averin & Voskoboyev, 1982). However, it is controversial whether it is energy dependent (Averin & Voskoboyev, 1982) or energy independent (Komai & Shindo, 1974*a*). Moreover, no investigations have been carried out in human erythrocytes.

The aim of the present study was to investigate the main features of thiamin transport in intact human erythrocytes and to differentiate between cytoplasmic and membrane events by the use of ghosts.

In rat erythrocytes, thiamin may bind to proteins (Voskoboyev & Averin, 1983) and may be phosphorylated intracellularly to thiamin-pyrophosphate by a thiamin-pyrophosphokinase which is present exclusively in the cytoplasm (Smits & Florijn, 1950; Deus & Blum, 1970). Since both events may influence the rate of thiamin uptake in humans as well, we also investigated thiamin phosphorylation by determining erythrocyte thiamin phosphate content after incubation, as well as thiamin binding in both erythrocytes and ghosts by using a microdialysis procedure.

A preliminary partial account of this work was presented at the 39th Congress of the Italian Physiological Society (Casirola et al., 1989).

Materials and Methods

Erythrocyte Preparation

Erythrocytes were prepared from bank blood (less than 10 days old) stored at 4°C in a citrate buffer (pH 7.4) containing glucose and adenine. After centrifugation at 12,000 × *g* in the cold, the plasma and the buffy coat were removed by suction and the cells were washed three times in cold saline and centrifuged.

Ghost Preparation

Right-side-out, pink ghosts resealed to contain 145 mM NaCl (Na^+ ghosts) or 145 mM KCl (K^+ ghosts) were prepared from the above-mentioned erythrocytes according to the method of Schwoch and Passow (1973), with minor modifications, just before use. The method is based on the reversible lysis of erythrocytes under controlled conditions of osmolarity, temperature and pH. As an index of the resealing extent, the hemoglobin content of ghosts was determined with a cyanmethemoglobin method (Kit Hemoglobin no. 125482, Boehringer Mannheim, Mannheim, FRG) and found to be consistently about 3% of that of initial erythrocytes.

Unless otherwise stated, Na^+ ghosts were used for all experiments.

Uptake Measurements

Incubations of erythrocytes and ghosts were carried out at 37 or 20°C in Tris-HCl buffer (pH 7.4) containing tritiated thiamin ([^{3}H]-T), under different experimental conditions (*see* figure legends). Incubation was started by adding 100 μl of erythrocytes or ghosts (in both cases hematocrit value was 0.5) suspended in thiamin-free incubation medium to 100 μl of the same medium containing appropriate concentrations of [^{3}H]-T. Incubations were stopped by transferring the samples to an ice-cold bath and by adding to each sample 1 ml of ice-cold isotonic thiamin-free medium. Erythrocytes or ghosts were immediately centrifuged at 12,000 × *g* (Beckman microfuge 12, Beckman, Fullerton, CA) for 30 and 60 sec, respectively, washed three times with cold, thiamin-free incubation medium and centrifuged again as described above. These washings allowed the complete removal of all the [^{3}H]-T present in the water adherent to the cells, as shown in preliminary experiments (*data not shown*). The total amount of radioactivity taken up by cells or ghosts was determined by means of a liquid scintillation counter (Packard Tri-Carb 2,000 CA analyzer, Packard Instrument Co., Downers Grove, IL) using 0.5 ml of their 10% trichloroacetic acid extracts obtained after centrifugation. All results were expressed as pmol of [^{3}H]-T · μl^{-1} of intracellular water, which was determined according to Speizer, Haugland and Kutchai (1985).

Thiamin Compounds in Erythrocytes

After 30 min incubation at 37°C with 0.2 μM [^{3}H]-T dissolved in a medium containing (in mM): 140 NaCl, 5 KCl, 2 $MgSO_4$, 15 Tris-HCl, pH 7.4, the erythrocytes were separated by centrifugation (20,000 × *g*) in the cold, frozen (−20°C) and thawed three times in a 1% Triton X-100 aqueous solution, and then deproteinized with 7% trichloroacetic acid. After centrifugation in the cold at 20,000 × *g*, the protein-free supernatant was purified and the content of thiamin and its phosphoesters determined according to the electrophoretic micromethod of Patrini and Rindi (1980).

Microdialysis

Six samples of erythrocytes and their corresponding freshly prepared ghosts were incubated at 37°C for 30 min with 0.2 μM [^{3}H]-T as described above. After incubation, all samples were separated by centrifugation and washed as indicated. Half of the samples of erythrocytes and ghosts were immediately deproteinized with 10% trichloroacetic acid, and the extracts counted radiometrically as described above. The remaining samples, after addition of 250 μl of thiamin-free incubation medium, were frozen and thawed as described for T compounds determination. The completely lysed samples were then dialyzed according to the microprocedure of Lau and Fujitaki (1981) against thiamin-free incubation medium for 24 hr at 4°C with continuous stirring. The samples were then deproteinized with 10% trichloroacetic acid, centrifuged and the supernatants counted radiometrically. Radioactivity contents were expressed as pmol of [^{3}H]-T per 100 μl of initial packed erythrocytes or ghosts.

Materials

[^{3}H]-thiamin (sp act: 7.5 Ci/mmol; 95% purity, as evaluated by descending paper chromatography with butanol : acetic acid : water = 120 : 30 : 50) was purchased from the Radiochemical Centre, Amersham (England). Unlabeled thiamin chloride hydrochloride and Amprolium were generous gifts of Prodotti Roche, Milan, Italy, and Merck Sharp and Dohme, Pavia, Italy, respectively. Pyrithiamin bromide hydrobromide, 4′-oxythiamin chloride and valinomycin were obtained from Sigma Chemical Co., Saint Louis, MO. All other chemicals were of analytical grade.

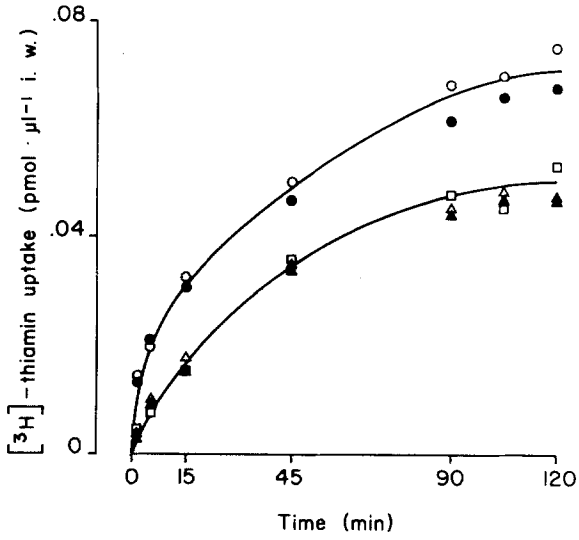

Fig. 1. Time course of thiamin uptake by human erythrocytes and ghosts. Uptake was measured at 37°C in the presence of 0.2 μM [^{3}H]-thiamin. Incubation medium contained (mM): 140 NaCl, 5 KCl, 2 $MgSO_4$, 15 Tris-HCl, pH 7.4: ○, erythrocytes; □, Na^+ ghosts; △, K^+ ghosts. The same medium with Na^+ replaced by K^+: ●, erythrocytes; ▲, K^+ ghosts. Symbols represent means of triplicate determinations for each of at least five different experiments. SEMs were within 10% of the mean values. *i.w.*, intracellular water

Results

Time Course of Thiamin Uptake

The uptake of [^{3}H]-T by human erythrocytes at 37°C was greater than [^{3}H]-T uptake by ghosts, Na^+ and K^+ ghosts showing the same uptake rate. In both systems the uptake was Na^+ independent and showed a monotonic increase with time, without reaching a clear equilibrium within 60 min (Fig. 1).

Uptake at Different Thiamin Concentrations

When erythrocytes were incubated at 37°C with different initial concentrations of [^{3}H]-T, a biphasic course was constantly observed which was nonlinear at low concentrations (approximately from 0.1 to 0.5 μM), and linear at higher concentrations. The

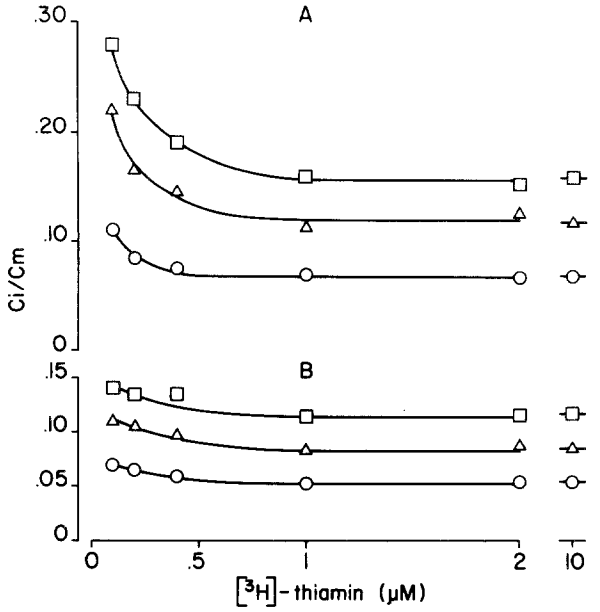

Fig. 2. Relationship between thiamin uptake by human erythrocytes and thiamin concentration in the medium. Thiamin uptake was measured after (○) 3, (△) 15 and (□) 30 min of incubation with different [^{3}H]-thiamin concentrations. Incubation medium contained (mM): 140 NaCl, 5 KCl, 2 $MgSO_4$, 15 Tris-HCl, pH 7.4. Incubation temperature: (*A*) 37°C; (*B*) 20°C. Results are expressed as the ratios of thiamin concentration in the cell to that in the medium. Symbols represent means of triplicate determinations for each of at least five different experiments. C_i, C_m correspond to [^{3}H]-thiamin concentrations inside erythrocytes and in the medium, respectively

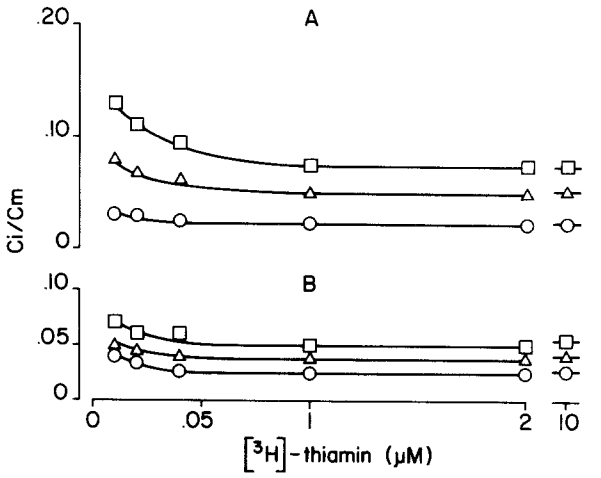

Fig. 3. Relationship between thiamin uptake by human erythrocyte ghosts and thiamin concentration in the medium. Thiamin uptake was measured after (○) 3, (△) 15 and (□) 30 min of incubation with different [^{3}H]-thiamin concentrations. Incubation temperature: (*A*) 37°C, (*B*) 20°C. Incubation medium, representation of results and number of experiments for each symbol were as in Fig. 2. C_i, C_m as in Fig. 2

biphasic course was more pronounced with increasing incubation time, and was virtually cancelled by lowering the incubation temperature to 20°C (Fig. 2). A similar biphasic course (albeit less pronounced) and a similar temperature sensitivity were found for ghosts (Fig. 3). When thiamin concentrations were plotted against rates of thiamin uptake, the cumulative uptake curves could be resolved into two components according to the procedure of Casirola et al. (1988): a linear component, expression of a non-saturable uptake system, and a hyperbolic component, expression of a saturable mechanism which exhibited Michaelis-Menten-like kinetics (Figs. 4 and 5). The apparent kinetic constants of the saturable component were calculated by computerized nonlinear regression (Graphpad package: ISI 1987) using as "initial estimates" values obtained by the method of Cornish-Bowden and Eisenthal (1978). The final values were: for erythrocytes, K_m = 0.12, 0.11 and 0.10 μM, and J_{max} = 0.01, 0.02 and 0.03 pmol · μl^{-1} of intracellular water after 3, 15, and 30 min incubation times, respectively, at 37°C; for ghosts, K_m = 0.16 and 0.51 μM, and J_{max} = 0.01 and 0.04 pmol · μl^{-1} of intracellular water after 15 and

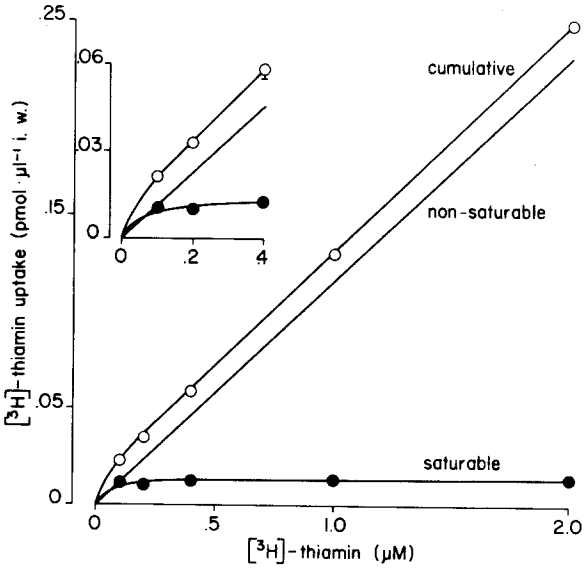

Fig. 4. Relationship between thiamin uptake by human erythrocytes and thiamin concentration in the medium. Thiamin uptake was measured after 15 min incubation at 37°C with different [^{3}H]-thiamin concentrations. Incubation medium was as in Fig. 2. Open symbols represent experimental data points (means of triplicate determinations for each of at least five different experiments). The curve of cumulative transport is the best-fit least squares regression (*see* Results). *Inset:* Initial part of the curve shown in detail with an expanded scale. Bars represent the SEMs. When not shown, bars are within symbol area. *i.w.* as in Fig. 1

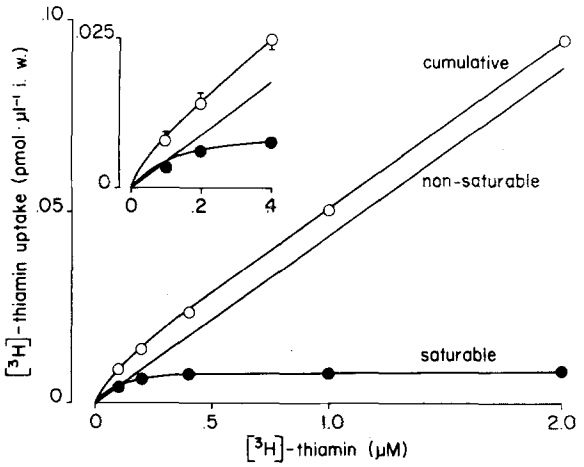

Fig. 5. Relationship between thiamin uptake by human erythrocyte ghosts and thiamin concentration in the medium. Experimental conditions, incubation medium, representation of results, number of experiments for each symbol and inset were as in Fig. 4. *i.w.* as in Fig. 1

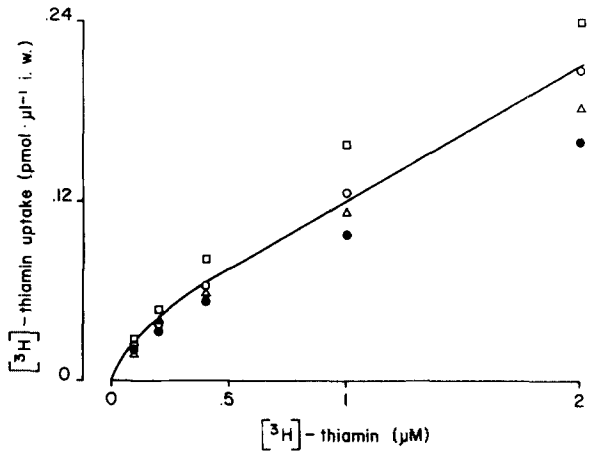

Fig. 6. Effect of D-glucose and metabolic inhibitors on thiamin uptake by human erythrocytes. Thiamin uptake was measured at 37°C after 30 min incubation with [^{3}H]-thiamin at different concentrations in the absence (○) and in the presence of: (●) 4.4 mM D-glucose; (△) 100 µM DNP; (□) 10 mM NaF. Incubation medium and number of experiments for each symbol were as in Fig. 2. SEMs were within 10% of the mean values. *i.w.* as in Fig. 1

30 min incubation, respectively, at 37°C. The curves obtained from the experimental data after 15 min incubation time for erythrocytes and ghosts, respectively, are reported as an example in Figs. 4 and 5. Data are not shown for the 3 and 30 min incubation times.

Effect of Glucose and Metabolic Inhibitors on Thiamin Uptake

The effect of metabolic energy on thiamin uptake by erythrocytes was assessed by incubating the cells with D-glucose or some metabolic inhibitors (NaF, DNP) at appropriate concentrations. Incubations were carried out for 30 min in media containing different [^{3}H]-T concentrations with or without D-glucose or inhibitors. No statistically significant differences (variance analysis) were observed (Fig. 6).

Specificity of Transport

In order to assess the specificity of the thiamin uptake mechanism, unlabeled thiamin and several thiamin analogues were used with [^{3}H]-T. Incubations of erythrocytes or ghosts were carried out at 37°C for 15 min after addition of different amounts of each compound to an incubation medium containing 0.2 µM [^{3}H]-T. In these conditions, the saturable component accounted for approximately 29 and 42% of the cumulative transport by erythrocytes and ghosts, respectively (*see* Figs. 4 and 5).

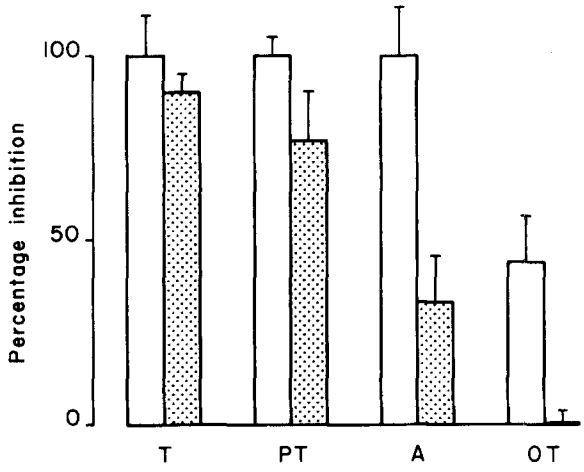

Fig. 7. Potency of unlabeled thiamin and thiamin analogues in inhibiting saturable thiamin transport in human erythrocytes and ghosts. Thiamin uptake was measured at 37°C for 15 min in the presence of 0.2 µM [^{3}H]-thiamin. Unlabeled thiamin and analogues were added to the incubation medium at an initial 2 µM concentration. Incubation medium was as in Fig. 2. Bars represent means of percentage inhibition ± SEMs of five different experiments. □, erythrocytes; ▨, ghosts. *T*, unlabeled thiamin; *PT*, pyrithiamin; *A*, amprolium; *OT*, 4′-oxythiamin

When the analogue : thiamin molar concentration ratio was 10 (Fig. 7), the saturable component of erythrocyte uptake was inhibited completely by unlabeled thiamin, pyrithiamin and amprolium, and only partially (44%) by oxythiamin. At the same

molar concentration ratio, unlabeled thiamin, pyrithiamin and amprolium inhibited ghost saturable uptake by 90, 77 and 33%, respectively, while oxythiamin was completely inactive (Fig. 7).

In preliminary experiments with a molar concentration ratio of 1, unlabeled thiamin, pyrithiamin, amprolium and oxythiamin were found to inhibit saturable [^{3}H]-T uptake by 67, 100, 89 and 0%, respectively, in erythrocytes, and by 34, 66, 0 and 0%, respectively, in ghosts.

Thiamin Compounds in Erythrocytes

After 30 min incubation with 0.2 μM [^{3}H]-T at 37°C, erythrocytes were found to contain mainly [^{3}H]-T (77.5% of total labeled thiamin), together with small amounts of thiamin-phosphoesters (18.7% thiamin-pyrophosphate and 3.9% thiamin-monophosphate). No labeled triphosphate was detected.

Binding or Translocation

To differentiate between [^{3}H]-T protein-binding and translocation to the intracellular or ghost space, previously incubated erythrocytes or ghosts were lysed and exposed to microdialysis (*see* Materials and Methods). The proportion of [^{3}H]-T found to be non-diffusible (protein-bound) after microdialysis was similar for erythrocytes and ghosts and accounted for about 20% of the total [^{3}H]-T present in the incubated elements (Fig. 8).

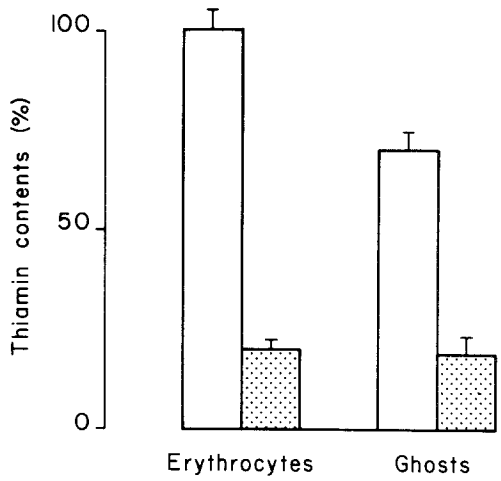

Fig. 8. Contents of total and protein-bound thiamin in human erythrocytes and ghosts. [^{3}H]-thiamin contents were measured after incubation at 37°C for 30 min in the presence of 0.2 μM [^{3}H]-thiamin: □, before and ▨, after microdialysis of hemolysed samples (*see* Materials and Methods). Incubation medium was as in Fig. 2. Bars represent means ± SEMs of five different experiments. 100% = thiamin content (2.75 ± 0.16 pmol · 100 μl^{-1} packed erythrocytes) of erythrocytes before microdialysis

Effect of Transmembrane Electrical Potential

The effect of membrane potential on the cationic [^{3}H]-T influx was evaluated by imposing an electrical potential across the erythrocyte or ghost membrane. To this purpose, two methods were used: (i) valinomycin-induced K^+ diffusion electrical potential, and (ii) anion substitution (Said & Redha, 1988; King & Gunn, 1989). In the first method, the uptake of 0.2 μM thiamin was evaluated in the erythrocytes and K^+ ghosts in the presence of the valinomycin, which caused an outward-directed K^+ gradient since the incubation medium contained a low K^+ concentration (5 mM). In the second method, the uptake of 0.2 μM thiamin was evaluated in Na^+ ghosts in the presence of anions with different lipid solubility ($SCN^- > SO_4^{2-}$). Incubation with the relatively lipid-soluble anion SCN^- creates a relatively greater negative intracellular compartment as compared to the poorly lipid-soluble anion SO_4^{2-}, a change which would be expected to affect any electrogenic component of thiamin transport. No statistically significant differences (variance analysis) in [^{3}H]-T uptake were observed by using valinomycin or SCN^- as compared to controls (absence of valinomycin or presence of SO_4^{2-} instead of SCN^-, respectively) (Figs. 9 and 10).

Discussion

The features of thiamin uptake by human erythrocytes found in this study are similar to those previously reported for rat erythrocytes (Komai & Shindo, 1974*a*; Averin & Voskoboyev, 1982). This process did not require cellular metabolic energy (Fig. 6), a finding which is in keeping with those of several authors using thiamin, amino acids and purines (Eavenson & Christensen, 1967; Loo et al. 1969; Komai & Shindo, 1974a). It exhibited a dual (saturable and nonsaturable) mechanism (Fig. 4), as shown also for small intestinal intact tissue and brush border vesicles (*see* Casirola et al., 1988), and for hepatic and cerebral tissue (Yoshioka, 1984; Patrini et al., 1988). The saturable component was especially important at low thiamin concentrations (<0.5 μM), followed Michaelis-Menten kinetics, was sensitive to incubation temperature (Fig. 2) and could be completely inhibited by unlabeled thiamin

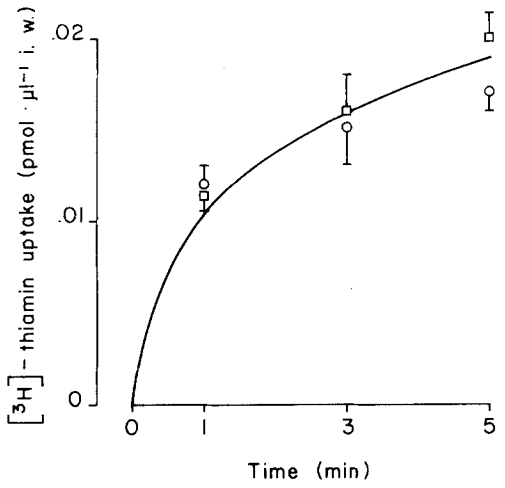

Fig. 9. Effect of valinomycin-induced K^+ diffusion transmembrane potential on the transport of 0.2 μM cationic [^{3}H]-thiamin in human erythrocytes. Uptake was measured at 37°C in the presence of (○) 20 μM valinomycin in 0.3% ethanol or (□) 0.3% ethanol (control). Incubation medium and number of experiments for each symbol were as in Fig. 2. Symbols represent means ± SEMS. *i.w.* as in Fig. 1

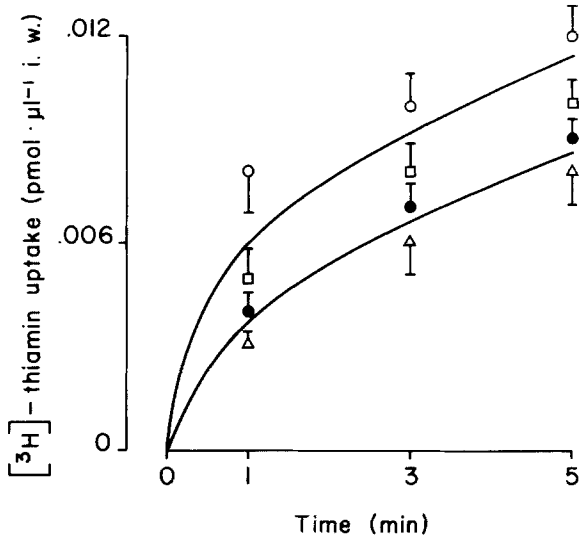

Fig. 10. Effect of membrane potential on the transport of 0.2 μM cationic [^{3}H]-thiamin in human erythrocyte ghosts. Negative membrane potential was imposed by using two different methods: (i) K^+ ghosts incubated at 37°C in the presence of (○) 20 μM valinomycin in 0.3% ethanol or (□) 0.3% ethanol (control); (ii) Na^+ ghosts incubated at 37°C in the presence of anions with different liposolubility osmotically substituted for Cl^- in the incubation medium: (●) SCN^-; (△) SO_4^{2-} (control). Other components of incubation medium and number of experiments for each symbol were as in Fig. 2. Symbols represent means ± SEMS. *i.w.* as in Fig. 1

and some thiamin structural analogues at relatively low concentrations (Fig. 7). The thiamin taken up by the cells was only partially (18.7%) phosphorylated to thiamin pyrophosphate.

The uptake process reflects primarily a true translocation into the intracellular space, since in microdialysis studies only 20% of thiamin entering the cell was found to be bound to membrane or intracellular protein (Fig. 8).

A thiamin-binding protein has been purified recently from rat erythrocytes. This protein, which has a high affinity for thiamin and a molecular weight of 32,000, accounts for only a very small proportion of the total intracellular protein content (Voskoboyev & Averin, 1983), a finding that appears to be consistent with the present results.

The kinetic parameters of the saturable component of erythrocyte thiamin transport could be reliably characterized. After 3, 15 and 30 min incubation time at 37°C, apparent K_m values were: 0.12, 0.11 and 0.10 μM and apparent J_{max} values were 0.01, 0.02 and 0.03 pmol · μl^{-1} of intracellular water, respectively. Thus, the saturable transport of erythrocytes seems to have an affinity for thiamin which is similar to that of rat isolated enterocytes and higher than that of rat isolated hepatocytes (*see* Table). Since in man the physiological levels of thiamin in plasma or serum are 0.01–0.02 μM (Weber & Kewitz, 1985; Bettendorff et al., 1986; Bötticher & Bötticher, 1987), thiamin transport into the erythrocytes appears to be accounted for almost exclusively by the saturable mechanism.

As far as transport capacity is concerned, erythrocytes transported thiamin at a rate which was 150 times and 600 to 10,000 times as low as that of rat isolated enterocytes and hepatocytes, respectively (Table). Since the hepatocyte has more transport sites than the erythrocyte, it appears to be particularly suitable for taking up a great amount of thiamin from plasma, an observation which can be related to the well-known function of the liver as a storage site for thiamin (Pearson, 1967).

Another feature of the erythrocyte transport of thiamin, which dissociates as a monovalent cation at pH 7.4 (Komai & Shindo, 1974*b*), is that the uptake is not affected by a relatively negative intracellular space (Fig. 9), suggesting that transport is most probably an electroneutral process.

As a whole, the present results indicate that at physiological plasma concentrations thiamin is transported into human erythrocytes by a high affinity and low capacity carrier-mediated electroneutral process, which is Na^+- and energy-independent and probably involves a facilitated diffusion.

The use of right-side-out resealed ghosts, which minimize the influence of the internal constituents

Table. Kinetic parameters of thiamin transport in different preparations in vitro

Preparation	Species	K_m (μM)	J_{max}	Source
Erythrocytes	Man	0.10–0.12	0.01 pmol · mg^{-1} protein · min^{-1}	This paper
Isolated enterocytes, normal	Rat	0.18	1.38 pmol · mg^{-1} protein · min^{-1}	Ricci & Rindi, 1989
Erythrocytes	Man	0.10–0.12	0.04 pmol · 10^{-6} cells · min^{-1}	This paper
Isolated hepatocytes	Rat	34.1[a] 1.26[b]	416[a] pmol · 10^{-6} cells · min^{-1} 24.2[b] pmol · 10^{-6} cells · min^{-1}	Yoshioka, 1984
Erythrocyte ghosts	Man	0.16–0.51	0.36 pmol · mg^{-1} protein · min^{-1}	This paper
Small intestinal microvillous vesicles	Rat	0.80	5.25 pmol · mg^{-1} protein · min^{-1}	Casirola et al., 1988
Isolated enterocytes, de-energized	Rat	0.48	0.84 pmol · mg^{-1} protein · min^{-1}	Ricci & Rindi, 1989
Blood-brain barrier	Rat	0.40–0.60	17.6–18.6 pmol · g^{-1} · min^{-1}	Greenwood et al., 1982
Everted jejunal sacs	Rat	0.16–0.63	865 pmol · g^{-1} · min^{-1}	Ventura et al., 1969; Hoyumpa et al., 1975
Everted intestinal rings	Rat	0.42	107 pmol · g^{-1} · min^{-1}	Akiyama et al., 1981

[a,b] Low and high affinity entry process, respectively.
For comparative purposes, all J_{max} values not originally expressed per min^{-1} were recalculated by assuming a linear velocity-time relationship; quantity units were re-expressed as pmol. For erythrocyte and ghost J_{max} recalculations, the amount of nonhemoglobin protein per cell volume and cell counts were determined experimentally (protein: Lowry et al., 1951; cell count: Sysmex Microcellcounter CC 120-Gelman Instrument, Milan, Italy).

and metabolism of the erythrocyte, allowed a separate study of the membrane mechanisms of the uptake process. In general, the resealed ghosts, while transporting thiamin to a lesser extent, retained the same transport features as the intact erythrocytes (Figs. 3 and 5). The saturable component at low thiamin concentrations accounted for 42% (Fig. 5) of cumulative transport as compared to 29% for erythrocytes, had similar affinity and capacity for thiamin, was less inhibited by the same molecular ratio of unlabeled thiamin and structural analogues (in particular amprolium and oxythiamin) (Fig. 7), and was electroneutral (Fig. 10). Only 20% of the transported thiamin was membrane bound (Fig. 8).

Since both small intestinal microvillous vesicles and resealed ghosts are vesicular membrane structures, it is interesting to compare their mechanisms for thiamin transport. Without considering differences in animal species (rat and man), both types of membranes appear to transport thiamin by a similar dual mechanism (for vesicles *see* Casirola et al., 1988), which is saturable at low (physiological) concentrations and nonsaturable at higher concentrations. In both membranes the saturable component displays affinities for thiamin of the same order of magnitude and similar molecular specificities, being inhibited to approximately the same extent by unlabeled thiamin and some structural analogues. However, transport capacities, even though not easily comparable owing to the different conditions of incubation, seem to be quite different, being lower for the ghosts (Table). These findings suggest that thiamin transport sites on both small intestinal brush border and erythrocyte membranes are structurally similar but different in number, being much fewer in the erythrocytes.

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