Energy Metabolism in Human Erythrocytes: The Role of Phosphoglycerate Kinase in Cation Transport

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Three models of disturbed erythrocyte metabolism, triose-depleted normal, phosphoglycerate kinase (PGK)-deficient, and pyruvate kinase (PK)-deficient cells, have been studied to examine further the role of PGK in erythrocyte cation transport. Sodium (Na^+) and potassium (K^+) transport were reduced only in cells fully depleted of triose. In such cells the PGK step presumably was inoperative due to total lack of substrate; 2,3-diphosphoglycerate (2,3-DPG) then became the sole substrate source for the remaining steps in glycolysis. At increased intracellular Na^+ concentrations which normally stimulate transport and glycolysis, triose-depleted cells had marked impairment of cation transport and ouabain-inhibitable lactate and pyruvate production from 2,3-DPG. PGK-deficient cells and normal cells with high intracellular Na^+ concentrations had similar increases in transport and ouabain-inhibitable lactate production. PK-deficient cells with high intracellular Na^+ concentrations showed an appropriate increase in transport but less stimulation of lactate production. Transport was not related to total cellular adenosine triphosphate (ATP) concentration. These data suggested that normal coupled cation transport occurred despite diminished metabolite flow through PGK, as in PGK- or PK-deficient cells. Transport was diminished only in triose-depleted cells where metabolite flow through PGK was presumably absent. These data, therefore, support the concept that transport and glycolysis interact at the PGK step, although impairment of PGK must be profound before its effect on transport is evident.

ACTIVE TRANSPORT of Na^+ and K^+ is linked with erythrocyte glycolysis. Cation transport is mediated by an adenosine triphosphatase (ATPase) which releases adenosine diphosphate (ADP) as a product of the hydrolysis of ATP.[1] The concentration of ADP influences the rate of glycolysis at the PGK reaction.[2-4] Consequently, when cation transport is accelerated, the rate of ADP production is increased, stimulating glycolysis. The increment in glycolysis is ouabain sensitive, confirming the dependence of the increment on an increase in $Na^+ - K^+$ transport.

We have previously studied metabolism and transport in erythrocytes which were depleted of their proximal glycolytic substrates (including triose phosphates) and which depended solely on the degradation of 2,3-DPG for the gen-

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eration of ATP.[5] Since substrate for the PGK step in glycolysis is zero in triose-depleted cells, they provide a means by which the importance of PGK in cation transport can be assessed.

In the present report, we have evaluated the role of PGK in cation transport by studying net changes in Na^+ and K^+ transport in normal, triose-depleted normal, PK- and PGK-deficient erythrocytes.

MATERIALS AND METHODS

Blood samples from normal persons and patients with erythrocyte PK and PGK deficiency were anticoagulated with preservative-free heparin (0.1 mg/ml). The red blood cells were separated and washed as previously described.[5] Cell counts were performed by standard methods[6] or in a Coulter Model S electronic particle counter. Assays for PGK and PK activity were performed by established techniques.[7]

Metabolic incubations and fresh red cell K^+ and Na^+ isotope flux studies were performed as previously described.[5] Specific activities of cells and media were determined at frequent intervals. In all studies reported here, Na^+ and K^+ transport were linear with respect to time; therefore the results are expressed as milliequivalents of Na^+ or K^+ transported per liter cells per hour. Oubain-inhibitable isotope flux was calculated from the ouabain-inhibitable component of total flux measured with $^{24}Na^+$ and $^{42}K^+$. Ouabain-induced net flux was determined by measuring the accumulation of Na^+ within the cells and loss of K^+ to the media induced by 0.1 mM ouabain. The differences between the isotope and net estimates of active Na^+ efflux and K^+ influx were taken as the values for the ouabain-inhibitable Na^+-Na^+ and K^+-K^+ exchange, respectively.

Perchloric acid filtrates prepared from the incubations were analyzed for ATP,[8] lactate,[9] 2,3-DPG,[10] and other glycolytic intermediates.[11] Substrate-depleted cells were prepared by washing three times in a glucose-free medium and incubating at 37°C for 1 hr. This procedure effectively eliminates glucose and the glycolytic intermediates above PGK.[5]

RBCs with increased intracellular Na^+ were prepared by incubation of the cells in a Krebs-Henseleit bicarbonate buffer with Na^+ adjusted to 50 meq/liter and potassium, 100 meq/liter, in the presence of 2 g/liter albumin and 5 mg/liter amphotericin B[12] (the same lot of drug was used in all studies). When the cells had acquired the desired internal Na^+ concentration (30–120 min), they were washed twice with the incubation buffer containing 10 g/liter albumin. This step was followed by a wash with standard Krebs-Henseleit buffer (sodium, 150 meq/liter; potassium, 5 meq/liter) and 2 g/liter albumin before resuspension and incubation.

Statistical analyses were performed by standard methods.[13] The slopes of the regression lines in Fig. 1–3 were determined by the method of least squares.

RESULTS

Table 1 offers a comparison between ouabain-inhibitable isotope flux and ouabain-induced net flux in normal and triose-depleted red blood cells. The difference between the net flux and isotope flux represents ouabain-inhibitable Na^+-Na^+ and K^+-K^+ exchange, respectively. When red blood cells were triose depleted (i.e., triose phosphates were immeasurable fluorometrically), Na^+-Na^+ exchange fell from 0.65 meq per liter cells per hr to 0.21 meq per liter cells per hr. In contrast, K^+-K^+ exchange rose from 0.14 meq per liter cells per hr to 0.38 meq per liter cells per hr, thus accounting for the apparent persistence of active K^+ transport in depleted cells when measured with $^{42}K^+$.

Ouabain-induced Na^+ accumulation and K^+ loss in normal red cells at normal and elevated internal Na^+ concentrations are shown in Fig. 1. The ratio of Na^+ to K^+ active transport was approximately 3:2. The results of nine studies performed in triose-depleted red blood cells at a variety of internal Na^+ con-

Table 1. Ouabain-inhibitable Cation Exchange

	n	Sodium (meq/liter cells/hr)*	Potassium (meq/liter cells/hr)*
Normal RBC			
Ouabain-inhibitable isotope flux	6	2.00 ± 0.23	1.31 ± 0.25
Ouabain-induced net flux	7	1.35 ± 0.11	1.17 ± 0.13
Ouabain-inhibitable exchange		0.65	0.14
Triose-depleted RBC			
Ouabain-inhibitable isotope flux	6	1.00 ± 0.12	1.03 ± 0.14
Ouabain-induced net flux	5	0.79 ± 0.06	0.65 ± 0.08
Ouabain-inhibitable exchange		0.21	0.38

*Mean ± 1 SD.

centrations are shown in these same figures are compared to normal. Active transport in depleted cells remained linear during the 3 hr of measurement. It is evident that triose-depleted red blood cells have marked diminution of active Na^+ and K^+ transport at high intracellular Na^+ concentrations. At normal intracellular concentrations the defect is present but less striking. Similar determinations for PGK- and PK-deficient red blood cells are shown in Fig. 2 and 3. These enzyme-deficient red blood cells increased Na^+ and K^+ transport normally in response to elevation of internal Na^+. Since there are no documented differences in membrane permeability of young and old RBCs, this apparent normal Na^+-K^+ transport in these mutant cells is not related to their relatively young mean cell age. K^+ measurements are less reliable (Fig. 3) in

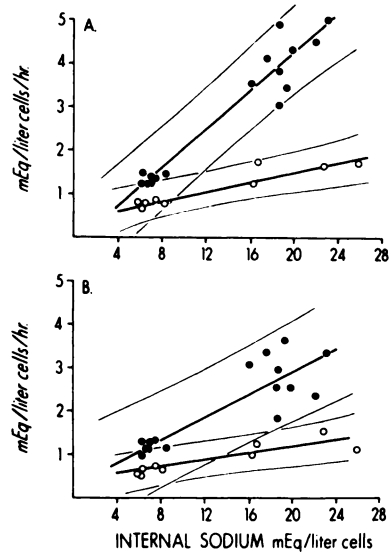

Fig. 1. Active Na^+ transport (A) and K^+ transport (B) in normal (●) and triose-depleted (○) red blood cells at various internal Na^+ concentrations. The figure depicts the linear regression and the 95% tolerance limits for Na^+ and K^+ active transport, respectively. The tolerance interval indicates the range of active transport rates at a given cell Na^+ concentration. The slope of the regression line for Na^+ transport in normal cells (0.220) is significantly different ($p < 0.05$) from that of triose-depleted cells (0.053). The slope of the regression line for K^+ transport in normal cells (0.140) is also significantly different ($p < 0.05$) from that of triose-depleted cells (0.040).

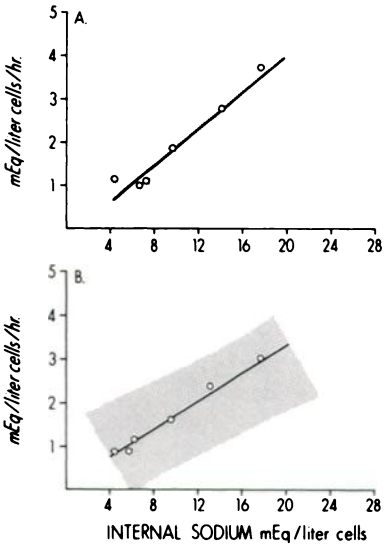

Fig. 2. Active Na^+ transport (A) and K^+ transport (B) in PGK-deficient red blood cells (o) at various internal Na^+ concentrations. The slopes of the regression lines for Na^+ and K^+ transport in PGK-deficient cells are 0.210 and 0.160, respectively. The shaded area represents the tolerance limits for transport over the same range of internal Na^+ concentrations in normal red blood cells, corresponding to Fig. 1.

the PK-deficient cells because of their inherent ouabain-insensitive K^+ leak to the media.[14]

Ouabain-inhibitable lactate production (Table 2) was increased in normal cells when the internal Na^+ was raised. It was similarly increased in PGK-deficient red blood cells under the same conditions. In contrast, PK-deficient red blood cells increased ouabain-inhibitable lactate production less than two-fold. The triose-depleted red blood cells were unable to increase glycolysis in response to elevated internal Na^+. 2,3-DPG and ATP levels did not fall in normal, PGK-, or PK-deficient red blood cells with normal or high internal Na^+ concentrations (Table 3). Both 2,3-DPG and ATP fell in the triose-depleted cells as previously described.[5]

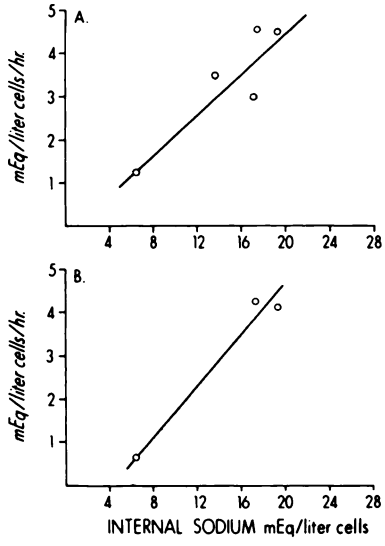

Fig. 3. Active Na^+ transport (A) and K^+ transport (B) in PK-deficient red blood cells (o) at various internal Na^+ concentrations. The slopes of the regression lines for Na^+ and K^+ transport in PK-deficient cells are 0.240 and 0.300, respectively. The shaded area represents the tolerance limits for transport over the same range of internal Na^+ concentrations in normal red blood cells, corresponding to Fig. 1.

Table 2. Lactate Production*

	n	Internal Sodium (meq/liter cells)	Total	Ouabain (mmoles/liter cells/hr)	Ouabain-inhibitable
Normal	6	7.0	1.93 ± 0.36	1.55 ± 0.34	0.38 ± 0.10
	4	19.7	2.61 ± 0.30	1.65 ± 0.14	0.96 ± 0.21
Triose-depleted*	4	7.0	0.70 ± 0.15	0.54 ± 0.08	0.16 ± 0.09
	4	19.4	0.59 ± 0.12	0.44 ± 0.09	0.15 ± 0.08
PGK-deficient	2	5.4	2.59, 2.93	2.37, 2.73	0.22, 0.20
	3	16.7	3.64 ± 0.04	2.81 ± 0.17	0.83 ± 0.14
PK-deficient	2	6.4	1.34, 1.35	0.98, 1.03	0.36, 0.32
	3	16.9	1.35 ± 0.10	0.86 ± 0.10	0.49 ± 0.08

*Lactate production is the sum of lactate plus pyruvate production in triose-depleted cells.

Table 3. ATP and 2,3-DPG Stability

	Internal Sodium	ATP (mmoles/liter cells)				2,3-DPG (mmoles/liter cells)			
		n	T_0	T_{60}	T_{180}	n	T_0	T_{60}	T_{180}
Normal	7.0	12	1.39 ± 0.21	1.36 ± 0.35	1.35 ± 0.12	4	4.35 ± 0.37	4.10 ± 0.45	4.40 ± 0.24
	19.7	4	1.13 ± 0.16	1.15 ± 0.14	1.12 ± 0.13	4	4.37 ± 0.36	4.33 ± 0.43	4.73 ± 0.54
Triose-depleted	7.0	4	0.97 ± 0.07	0.75 ± 0.08	0.65 ± 0.06	4	4.36 ± 0.35	3.69 ± 0.39	2.41 ± 0.36
	19.4	4	0.60 ± 0.06	0.35 ± 0.11	0.37 ± 0.07	4	4.37 ± 0.94	3.76 ± 0.36	2.17 ± 0.88
PGK-deficient	5.4	3	0.85 ± 0.12	0.78 ± 0.05	0.99 ± 0.01	3	8.11 ± 1.53	8.50 ± 2.84	8.18, 11.65
	16.7	2	0.46, 0.49	0.48,0.38	0.67, 0.58	2	5.79, 5.91	6.88, 7.11	7.73, 9.14
PK-deficient	6.4	2	1.11, 1.50	1.10	1.13	2	13.70, 13.97	13.70	14.77
	16.9	2	1.04, 1.17	1.04, 1.24	1.00, 1.00	2	13.72, 13.83	14.14, 15.09	13.58, 14.46

DISCUSSION

Reported studies of cation transport in glucose-depleted cells have suggested that ATP generated at PGK is integrally involved with cation transport.[2-4] Feig and his co-workers studied Na^+ and K^+ transport in triose-depleted cells in which PGK presumably was bypassed and 2,3-DPG was the sole energy source.[5] Using radioactive sodium ($^{24}Na^+$) and potassium ($^{42}K^+$), these authors noted that Na^+ transport was decreased more profoundly than K^+ transport. Sachs has emphasized that Na^+ and K^+ transport are closely coupled and that the persistence of apparent K^+ pumping in the triose-depleted cell is due to an artifact resulting from increased ouabain-inhibitable K^+-K^+ exchange.[15] This ouabain-inhibitable process is due to an increased exchange in triose-depleted cells of $^{39}K^+$ and $^{42}K^+$ across the cell membrane and thus is not associated with net transport. Studies presented here confirm coupling of Na^+ and K^+ transport and reveal increased ouabain-inhibitable K^+-K^+ exchange in depleted cells as previously shown by Glynn.[16] In addition, our studies show that ouabain-inhibitable Na^+-Na^+ exchange decreased in triose-depleted cells. This previously has been demonstrated only at low ATP values.[17] Since ouabain-inhibitable Na^+-Na^+ and K^+-K^+ exchanges interfere with the isotopic measurement of active cation transport, subsequent studies were accomplished by measuring the ouabain-induced net changes in cell $^{23}Na^+$ and medium $^{39}K^+$. The changes in Na^+ and K^+ are small and difficult to measure under normal conditions without the aid of isotopes. For this reason, it was desirable to stress

the transport system by increasing the intracellular Na^+ concentration. As cell Na^+ is increased, transport is stimulated,[18] and measurable ouabain-induced changes in cell and medium cation content occur. Amphotericin was used in these studies, since it causes a reversible change in membrane permeability, thus allowing one to prepare erythrocytes of a desired cation composition.[12]

These data support the concept that PGK is influenced by ADP production from cation transport. Triose-depleted cells are the only model in which no metabolite flows through PGK. In these cells, 2,3-DPG (4.4. m*M*) is substrate for the remaining steps of glycolysis. The enzyme 2,3-DPG phosphatase is responsible for the degradation of 2,3-DPG to 3-phosphoglycerate (3-PG). Since the activity of this enzyme is not influenced by ADP, the rate of 2,3-DPG degradation, and hence, lactate plus pyruvate production in triose-depleted cells, is not influenced by an increase in the cell Na^+ and stimulation of active transport. The impaired cation flux secondary to impaired metabolism in triose-depleted cells also suggests a preferential role for PGK-generated ATP in transport. Schrier has demonstrated that a fraction of red cell PGK is located on the membrane,[19,20] and the proximity of PGK to membrane-bound Na^+, K^+ ATPase plus the known interaction of ATPase-produced ADP on PGK[2-4] make this concept plausible. PGK-generated ATP, however, cannot be an exclusive source of ATP for transport, since considerable transport remains in the triose-depleted cells. Furthermore, elevation of the internal sodium stimulates transport in these cells, although the response is blunted. The source of ATP for this residual transport response is unclear and appears not to be from 2,3-DPG via PK, since ouabain-inhibitable lactate plus pyruvate production does not increase with an increase in the internal Na^+ concentration. Alternatively, a preformed PGK-generated ATP pool may exist and supply energy for transport, or transport may be sustained simply by the general cellular ATP pool.

PGK-and PK-deficient erythrocytes were consequently examined as models which might be similar to triose-depleted cells. However, in contrast to triose-depleted cells, PGK-deficient cells were capable of normal Na^+ and K^+ transport. One possible explanation for these findings is that the residual PGK activity in these cells (10%–15%) allows sufficient metabolite flow through PGK to maintain cation transport. Mitochondrial ATP production is probably insignificant in the PGK-deficient cell population, as fewer than 10%–15% of the cells are reticulocytes and NaCN (5 m*M*) had no effect on transport, ATP concentration, or lactate production in one study (data not shown). Decreased PGK activity was present in the isolated membranes as well as the cytoplasm of these mutant cells, so that disproportionately high membrane PGK activity cannot explain the normal cellular transport capability.

The distal glycolytic block seen in PK-deficient red blood cells will inhibit PGK by-product inhibition and thereby may decrease the synthesis of PGK-generated ATP. The magnitude of this inhibition may have been insignificant, since PK-deficient RBCs transported cations at normal rates when these cells were stressed by elevated intracellular Na^+. However, glycolysis in PK-deficient cells was minimally stimulated by the presence of elevated cell Na^+, suggesting that the ATP was supplied by mitochondrial activity in these reticulocyte-rich

cell preparations (60%–80%). Unfortunately, we were unable to incubate PK-deficient cells under N_2 or in the presence of NaCN to measure glycolysis without mitochrondrial activity and resolve this question, since rapid hemolysis occurred under these conditions.

The absolute concentration of cellular ATP seems to be less important for transport than the ability to produce new ATP. The high Na^+ PGK-deficient cell is able to sustain cation transport at an ATP concentration which is slightly less than that present in the triose-depleted normal cells which have diminished transport. This paradox of decreased cation transport in cells with relatively normal ATP suggests that cellular ATP may be compartmentalized;[21] only a fraction of the total may be available to, or be the preferential substrate for, the membrane ATPase. Studies to date at least suggest that the PGK-generated ATP, or perhaps the membrane-associated portion, is the preferential, but not exclusive, source of ATP for transport.

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