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RELATIONSHIP OF SOLUTE PERMEABILITY TO ERYTHROCYTE GLYCOLYSIS

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SUMMARY

Erythrocyte suspensions demonstrate remarkable changes in glycolytic rate in response to the permeability characteristics of the solutes present. Erythrocytes when placed in electrolyte or nonelectrolyte media of limited permeability produce lactate at accelerated rates, attributable to a stimulation of ATP degradation. The mechanism(s) of increased turnover of ATP and the purpose to which the excess energy is directed remain to be clarified.

INTRODUCTION

The human erythrocyte engages only in a limited metabolism directed to little other than its immediate survival. Energy production in these cells is dependent on a functional Embden–Meyerhof pathway as the sole source of ATP generation. Energy expenditure by the cells has been defined only to the extent of an estimated 15–20% of the basal glycolytic rate, which is regulated by and directed to active cation transport[1,2]. The purpose to which the remaining basal energy production is expended remains unknown. Investigation of factors serving to regulate glycolytic rate has been undertaken with the purpose of gaining insight into the nature of those energy-consuming processes fundamental to the survival of these cells. In the course of these studies a marked stimulation of the glycolytic rate was observed on suspending erythrocytes in media containing inert solute of low permeability. Regulation of this type has not been described previously and a significant work function may be implied. Studies were initiated to inquire further into the phenomenon.

MATERIALS AND METHODS

All experiments were carried out using red cells prepared from heparinized, freshly drawn human blood from healthy donors. The cells were washed three times with normal saline and the top layer of leucocytes discarded. The washed cells were added directly to reaction tubes at low concentration (2–5% suspension) to minimize changes in media pH. Incubations were at 37°, following which aliquots were removed

Abbreviation: TES, *N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid.

for hemoglobin analysis[3] and for de-proteinization with perchloric acid. The perchloric acid extracts were neutralized with KOH and assayed directly for one or more of the following: glucose, lactate, pyruvate, fructose diphosphate, triose phosphates and ATP. Assays were by enzyme-coupled pyridine nucleotide oxidation or reduction measured fluorometrically[4,5] except in the case of lactate (measured by $A_{340\ nm}$). In certain cases, cells were washed in saline following incubation prior to hemoglobin, K^+ and total nucleotide analyses. All incubation mixtures contained 20–25 mM *N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid (TES), adjusted to appropriate pH with NaOH, as buffer reagent. Maintenance of media pH was verified in all cases with changes of less than 0.1 pH unit recorded. Cellobiose (4-(β-D-glucosido)-D-glucose) was purchased (Calbiochem) as a chromatographically homogeneous product. Osmolarity of suspending media was standardized with the Fiske osmometer.

RESULTS

Suspending media and glycolytic rate

Stimulation of glucose metabolism of yeast cells[6] and of rat adipose tissue and diaphragm[7] has been reported in hyperosmotic media containing either excess salt or non-electrolyte. Definition of mechanism(s) relative to the observed stimulation remains to be clarified. Erythrocyte suspensions in NaCl media exhibit little differences in glycolytic rate whether in hypo-, iso- or hyperosmotic concentrations of the salt (Fig. 1). On the other hand, replacement of NaCl with an impermeable non-electrolyte such as cellobiose results in a remarkable acceleration of glycolytic rate, clearly unrelated to media hyperosmolarity as illustrated in Fig. 1. Erythrocyte suspensions were found to demonstrate maximal glycolytic rate in near iso-osmotic (300 mosM) media containing impermeable non-electrolyte (or electrolyte of low anion permeability, see later) as principal solute. Relative glycolytic rates of erythrocyte suspensions prepared in iso-osmotic mixtures of cellobiose and NaCl are shown in Fig. 2, with maximal rates

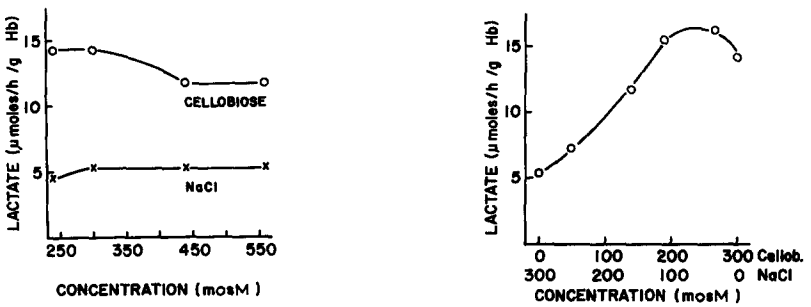
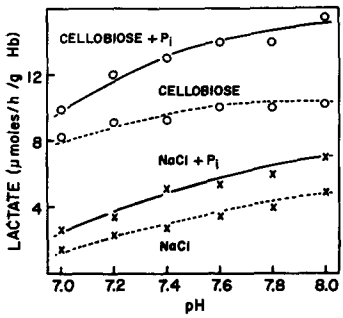


Fig. 1. Effects of tonicity and composition of suspending media on erythrocyte glycolytic rate. Incubation mixtures contained freshly drawn and prepared erythrocytes at 2.5%, 0.02 M TES buffer (pH 7.2), 1.2 mM Na_2HPO_4, 0.4 mM glucose, and NaCl or cellobiose in the indicated amounts. Lactate production was determined after 1 and 2 h incubation with the average hourly values shown. Lactate production was approximately linear with incubation time and hemolysis minimal in each of the tested media.

Fig. 2. Glycolytic rate of erythrocyte suspensions in iso-osmotic mixtures of NaCl and cellobiose. Incubation mixtures contained NaCl and/or cellobiose as indicated and erythrocytes, buffer, phosphate and glucose as described in the experiment shown as Fig. 1. Lactate production was determined after 1 and 2 h with the average hourly values shown.

observed in mixtures containing between 1/10 and 1/3 parts of salt. Erythrocyte suspensions in normal saline respond to elevation of medium pH (ref. 8) and P_i (ref. 9) by increased glycolytic rate in the manner illustrated in Fig. 3 (lower curves). Stimulation by pH and P_i has been attributed to their selective activation of phosphofructokinase[10–12] and to moderation by P_i of Glc-6-*P* inhibition of hexokinase[13]. Stimulation of glycolytic rate induced in cellobiose media exceeds that resulting from the action of pH and P_i to the extent illustrated in Fig. 3. Stimulation in cellobiose media demonstrates



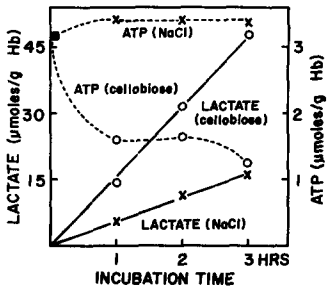


Fig. 3. Relative effects of pH, P_i and impermeable solute (cellobiose) on erythrocyte glycolytic rate. Incubation mixtures contained freshly drawn and prepared erythrocyte at 5%, 0.025 M TES buffer at indicated pH, 10 mM P_i (at appropriate pH) where indicated, 0.4 mM glucose and 300 mosM NaCl or cellobiose.

Fig. 4. ATP levels and glycolytic rates in cellobiose and NaCl media. Incubation mixtures contained 2.5% erythrocytes, 0.02 M TES buffer (pH 7.2), 1.2 mM phosphate, 0.4 mM glucose and 300 mosM NaCl or cellobiose where indicated. Aliquots were removed at the designated incubation times for lactate determinations and cellular ATP measure.

a general insensitivity to media pH between 7 and 8 with only slightly greater lactate production recorded at the higher pH. Stimulation of the glycolytic pathway in cellobiose medium and by pH and P_i demonstrate in common an accompanying accumulation of fructose diphosphate and triose phosphates (not shown), suggesting over-lapping effects with respect to phosphofructokinase stimulation[10–12].

Glycolytic stimulation and ATP

Accelerated rates of erythrocyte glycolytic flow induced in cellobiose media are accompanied by a characteristic, sharp drop in the intracellular steady-state level of ATP (Fig. 4). On the other hand, incubation in normal saline results in a steady, low rate of lactate production and maintenance of ATP at the initial high levels as shown (Fig. 4). From these findings, a mechanism is suggested in which enhanced glycolytic flow results in cellobiose medium as a consequence of increased ATP degradation, presumably induced as an indirect effect at the cell membrane level. Stimulation of glycolytic flow in cellobiose medium is maintained at a constant rate (see Fig. 4), reflecting a corresponding high rate of induced ATP turnover.

Effects of media composition and ATP degradation rate

Incubation of erythrocytes in the absence of added glucose and the presence of fluoride (and phosphate) avoids utilization and prevents resynthesis of ATP by glycolytic reactions. Incubation under these conditions, therefore, allows a measure of the existing rate of cellular ATP degradation (utilization) without interference by glyco-

lytic reactions. In Fig. 5 are shown comparative rates of ATP degradation in erythrocytes incubated without glucose in NaCl and sucrose media (cellobiose was avoided due to slight contamination by free glucose) containing added fluoride and phosphate.

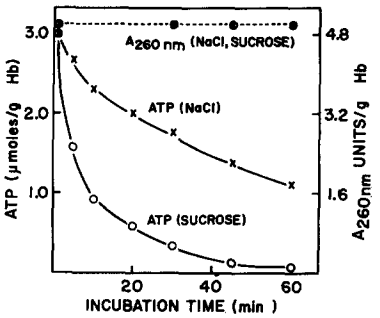


Fig. 5. Relative rates of ATP degradation in NaCl and sucrose media. Incubation mixtures contained 5% erythrocyte, 0.02 M TES buffer (pH 7.2), 5 mM NaF, 5 mM Na_2HPO_4 (pH 7.2) and NaCl or sucrose (300 mosM) as indicated. Aliquots were removed at the designated incubation times for lactate determinations, cellular ATP, and total nucleotide measure (latter by $A_{260\ nm}$).

Resynthesis of ATP by endogenous substrate (primarily 2,3-diphosphoglycerate) was effectively blocked in these experiments as evidenced by little or no accumulation of pyruvate or lactate (not shown). Further, since little loss of cell nucleotides occurred during the incubation period (see $A_{260\ nm}$ measure), decreases in ATP level may be presumed to reflect degradation only. On the basis of these results it is evident that ATP degradation proceeds more rapidly in cells suspended in the sucrose medium, thus accounting for increased glycolytic rates observed in this and similar media. The mechanism(s) involving increased ATP energy-drain induced in the sucrose medium and the nature of the work function to which it may be directed remain to be assessed.

Effect of suspending media on glycolytic rate, ATP and K^+

Incubation of erythrocytes in NaCl medium (pH 7.2) demonstrates a basal rate of lactate production accompanied by very slight reductions of cell ATP and K^+ (Table I). Addition of 1/10 part KCl to the NaCl medium results in a small increase in lactate production, maintenance of K^+ (by activation of the Na^+ pump[1,2]) and slight reduction in cell ATP. Incubation of cells in the presence of a variety of non-electrolytes of low permeability induces in each case, accelerated lactate production accompanied by reduction of cell ATP and loss of cell K^+. Incubation of cells in the presence of various sodium salts of known (or suspected) low anion permeability (in contrast to the highly permeable Cl^- (see ref. 14) also effects stimulation of lactate production and reduction of ATP with intermediate losses of cell K^+ recorded. Changes in cell K^+ were unaffected by ouabain addition to the various media examined (except in NaCl *plus* KCl medium). Among the medium solutes examined and listed in Table I are included several selected on the basis of their well-known use in blood preservation (citrate), plasma expansion (mannitol, sucrose), intravenous X-ray diagnosis (sodium iothalamate[15]) and as a buffer agent (glycylglycine). In view of likely irreversible deleterious effects which may be induced in the erythrocyte in the presence of the above solutes, their further study in this regard is anticipated.

TABLE I

EFFECT OF MEDIA COMPOSITION ON GLYCOLYTIC RATE, ATP AND K^+

Incubation mixtures contained freshly drawn and prepared erythrocytes at 5%, 0.025 M TES buffer (pH 7.2), 0.4 mM glucose and 300 mosM of the listed medium solute.

Medium solute	*Lactate produced per h (μmoles/g Hb)*	*Cell ATP after 2 h at 37° (μmoles/g Hb)*	*Cell K^+ after 2 h at 37° (mequiv/l)*
NaCl	2.8	2.64	85
NaCl + KCl (9:1)	4.1	2.26	93
Cellobiose	9.0	0.64	34
Sucrose	8.4	1.15	31
Lactose	10.2	1.19	34
Mannitol	8.2	0.96	31
Dulcitol	10.0	1.15	31
Trisodium citrate	8.5	0.51	68
Disodium tartrate	8.4	0.62	78
Sodium iothalamate	10.6	0.89	59
Glycylglycine (sodium salt)	10.8	1.19	67

DISCUSSION

Erythrocyte suspensions demonstrate profound changes in glycolytic rate in response to media composition. Aside from the well-known responses to media pH, P_i and cation composition, changes in glycolytic rate result in hyper-, hypo- and iso-osmotic media containing electrolyte or non-electrolyte of limited permeability. Although the observed stimulation of glycolytic rate in electrolyte media of low anion permeability and impermeable non-electrolyte media share similar characteristics, the question of identity of the mechanisms involved remains to be determined. Recent reports attributing a dependence of glycolytic rate on osmotic swelling and shrinking, with resultant changes in intracellular ATP concentration proposed as the probable primary regulatory factor[16,17], are not consistent with the present findings. In this respect, osmotic swelling and shrinking of cells effect only minimal changes in glycolytic rate when induced in hypo- and hypertonic NaCl media (see Fig. 1). The reported effect on glycolytic rate, attributed to media osmolarity[16,17], is more probably a consequence of the variable sucrose additions employed to alter media osmotic strength.

The observed stimulated glycolytic rates induced in media of low solute permeability would appear to be the result of an increased rate of ATP breakdown (utilization?), mediated directly or indirectly at the cell membrane level. The extent to which redistribution of ions between cell and media, as illustrated in the case of K^+ loss (see Table I) in the presence of impermeable non-electrolyte and electrolyte of low anion permeable, may be related to the observed increase in energy expenditure by the cells is being investigated currently. It has been known for a number of years[18,19] that marked shifts in ion distribution occur on placing erythrocytes in non-electrolyte media with alterations in cell pH (refs. 19, 20) and permeability to P_i (ref. 21) resulting. The extent to which these latter effects may contribute to the observed stimulation of

glycolysis induced on low-electrolyte media remain to be assessed further. It is clear, however, that stimulation in the presence of impermeable, non-electrolyte exceeds that which can be attributed to pH and P_i alone (see Fig. 3). Furthermore, although permeability effects observed in non-electrolyte media can be greatly modified by addition of small amounts of salts[19,20,22], stimulated glycolytic rates are maintained in cellobiose media in spite of considerable addition of NaCl as illustrated in Fig. 2.

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