Although no accurate assessment for the contribution of the cell wall to the mass of the cell has been possible, it may be appreciably larger than the value of about 15% suggested by analyses for glycosaminopeptide components using trypsin-treated walls. It is also apparent that the extent of action by EDTA on whole cells of *P. aeruginosa* cannot be properly assessed from experiments with isolated cell walls. These results and those of CORPE AND SALTON[9] emphasise the need for caution when using cell walls for the study of cell surfaces and particularly as a source of lipopolysaccharide.

This work was supported by a grant from the Medical Research Council (to N.A.R.). We are grateful to Mr. E. W. LOWN and Mr. F. BROWN for carrying out gas–liquid chromatographic separations, and to Mr. A. H. FENSOM for reference to his results.

Department of Chemistry,
The University,
Hull (Great Britain)

N. A. ROBERTS
G. W. GRAY
S. G. WILKINSON

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Received June 6th, 1967

Biochim. Biophys. Acta, 135 (1967) 1068–1071

BBA 73028

Effect of ouabain on deoxynucleoside metabolism in hereditary spherocytic human erythrocytes

The active movements of Na^+ and K^+ in the human erythrocyte are now known to be coupled to the activity of an ATP-hydrolyzing system (ATP phosphohydrolase, EC 3.6.1.4) located in the membrane, which is stimulated synergistically by Na^+ and K^+ (refs. 1, 2). Inhibition of active cation movements by the cardiac glycoside, ouabain causes a fall of 15% in the glycolytic rate when fresh erythrocytes are incubated *in vitro*[3], thus demonstrating that the activity of the membrane

$(Na^+ + K^+)$-stimulated ATPase is responsible for controlling this portion of glycolysis. It is probable that the connection between active cation movements and glycolysis is due to changes in ADP or P_i concentrations which result from the activity of the cation pump ATPase. However the identity of the glycolytic enzyme which is sensitive to such changes in ADP or P_i concentration is not established with certainty.

Phosphofructokinase (EC 2.7.1.11) has been proposed as the most likely site for stimulation of glycolysis by ATPase activity, either by the effect of a lowered ATP/ADP ratio on the enzyme or by a raised P_i concentration resulting from ATP hydrolysis[4,5]. Alternatively it has been suggested that the regulation may depend on the interaction of ATPase with phosphoglycerate kinase (EC 2.7.2.3) such that the ADP generated by the ATPase acts as a rate-limiting substrate for portion of the phosphoglycerate kinase in the erythrocyte[3]. These two alternatives may be distinguished by measuring the effect of ouabain on lactate production from deoxynucleosides. These compounds are converted to lactate by a pathway which joins the reactions of glucose degradation at the level of triose phosphate[6]; thus phosphofructokinase is not involved in deoxynucleoside metabolism. If the ATPase exerts its regulation at the phosphofructokinase level it would be expected that ouabain would be without effect on the lactate production from deoxyadenosine and deoxyinosine. Conversely if the ATPase regulates glycolysis through phosphoglycerate kinase then ouabain should reduce lactate production from deoxynucleosides.

When washed erythrocytes from normal donors were incubated with glucose *in vitro*, the addition of ouabain produced a fall in the rate of lactate production of 0.4–0.5 μmole/ml cells per h. However when deoxyadenosine was added as sole substrate, lactate production from this compound was not consistently inhibited by ouabain. For this reason an effect was sought using erythrocytes from patients with hereditary spherocytosis. These cells have an increased inward Na^+ leak, which in turn stimulates the active pumping of cations[7]. Spherocytes have greater amounts of glycolytic energy devoted to active cation transport and thus the inhibitory effect of ouabain on lactate production may be considerably magnified.

Blood was withdrawn into heparin anticoagulant from donors with proven hereditary spherocytosis and the erythrocytes washed four times in a saline medium to remove white cells and glucose. Reticulocyte counts in these donors ranged from 8 to 12%. The cells were then suspended at about 40% haematocrit in a saline medium of pH 7.45 and incubated for 1 h at 37° after addition of glucose (5 mM), adenosine, deoxyadenosine or deoxyinosine (each 15 mM). Aliquots were taken at 0 and 1 h incubation and deproteinized with $HClO_4$. The neutralized perchloric acid extracts were assayed for lactate using NAD and lactate dehydrogenase[8] and for glucose using glucose oxidase and *o*-dianisidine[9]. Duplicate samples always agreed to within $\pm$ 1.5% for both lactate and glucose.

Fresh hereditary spherocytes converted glucose to lactate at rates comparable with normal erythrocytes (3.0 μmoles lactate/ml per h; see Table I). When this abnormal blood was stored at 4° for 24 h prior to incubation, glycolysis was accelerated to almost double the normal rate (6.9 μmoles lactate/ml per h). In both instances glucose was almost solely converted to lactate, since more than 93% of the carbon atoms in the glucose utilized appeared as lactate. Thus the activity of the hexose monophosphate shunt is as low in hereditary spherocytes as it is in normal erythro-

TABLE I

INHIBITION OF GLYCOLYSIS BY OUABAIN IN HEREDITARY SPHEROCYTIC ERYTHROCYTES

Blood from donors with hereditary spherocytosis was used either fresh or after storage for 24 h at 4°. Washed erythrocytes were suspended at 40% haematocrit in a saline medium of composition 145 mM NaCl, 5 mM KCl, 10 mM glycylglycine, 30 mM imidazole (pH 7.45) *plus* 0.1% (w/v) human serum albumin and added to paired flasks containing glucose (5 mM), nucleoside or deoxynucleoside (15 mM) both in the presence and absence of ouabain (30 μM). Suspensions were incubated at 37° and at 0 and 1 h aliquots were taken and deproteinized in an equal volume of 6% (w/v) $HClO_4$, the supernatant was neutralized with KOH and analyzed for lactate.

Substrate	*Fresh cells*		*Stored cells*	
	Lactate production (μmoles/ml cells per h)	*Inhibition by ouabain*	*Lactate production (μmoles/ml cells per h)*	*Inhibition by ouabain*
Glucose	3.0	0.6	6.9	0.95
Adenosine	2.75	0.55	5.5	1.05
Deoxyadenosine	2.3	0.2	4.3	0.4
Deoxyinosine	2.2	0.3	3.6	0.6

cytes. Addition of ouabain (30 μM) to the suspensions inhibited lactate production by 0.6 μmole/ml per h in fresh spherocytes and by 0.95 μmole/ml per h in stored spherocytes, while glucose utilizations were correspondingly decreased by half these amounts. The greater inhibition of glycolysis by ouabain in the stored spherocytes indicates that the active cation pump had an increased demand for metabolic energy probably due to the greater Na^+ content of these cells. Hereditary spherocytes also utilized adenosine at rates comparable to glucose and the addition of ouabain reduced the lactate production from adenosine by an amount similar to that observed with glucose as substrate.

Deoxynucleosides were converted to lactate by hereditary spherocytes at rates somewhat less than that observed for the corresponding nucleosides. Ouabain inhibited this lactate production from deoxynucleosides by 0.2–0.3 μmole/ml per h in fresh hereditary spherocytes and 0.4–0.5 μmole/ml per h in the stored hereditary spherocytes (Table I). Further experiments with both fresh and stored hereditary spherocytes have confirmed the inhibition of deoxynucleoside metabolism by ouabain and established that the reduction of lactate production is highly significant ($P < 0.001$, $n = 11$).

This result thus shows that inhibition of the ($Na^+ + K^+$)-stimulated ATPase reduces glycolysis by an action on some enzyme common to both the pathways of glucose and deoxynucleoside metabolism. Clearly phosphofructokinase cannot be the regulator of glycolysis under the conditions of these experiments. Phosphoglycerate kinase is left as the most likely enzyme whose rate can be regulated by activity of the ($Na^+ + K^+$)-stimulated ATPase of the erythrocyte membrane. PARKER AND HOFFMAN[10] have reached similar conclusions by demonstrating that the activity of phosphoglycerate kinase bound to the erythrocyte stroma was sensitive to ouabain only when ATP was also present to activate the membrane ATPase. Since some of the phosphoglycerate kinase of the erythrocyte is associated with the stroma[11], this interaction between ATPase and phosphoglycerate kinase may be brought about by these two enzymes being spatially adjacent on the membrane. Even if the two enzymes are not closely spaced, a membrane pool of ADP formed

by ATPase may serve to link the activity of the two enzymes (see ref. 10). Whatever the mechanism of coupling of this glycolytic enzyme to ATPase its ultimate result is that energy production from glycolysis is varied appropriately to the energetic demands of active cation transport in the erythrocyte.

This work was supported by a grant from the National Health and Medical Research Council of Australia.

Department of Medicine,
University of Sydney,
and Clinical Research Unit,
Royal Prince Alfred Hospital,
New South Wales (Australia)

J. S. WILEY

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Received August 7th, 1967

Biochim. Biophys. Acta, 135 (1967) 1071–1074

BBA 73025

Some biochemical and X-ray diffraction studies of mitochondrial outer membrane

During the preparation of mitochondrial outer membrane for X-ray diffraction studies, it was observed that mild sonication stripped off a large proportion of the marker enzymes monoamine oxidase[1] and rotenone-insensitive NADH:cytochrome *c* oxidoreductase[2]. The diffraction experiments and parallel electron microscopy studies indicated that the thickness of the outer membrane in a hydrated state is of the order of 115 Å and that there is a small change (detectable at the dried state) in membrane dimension associated with removal of protein by sonication.

Preparation of outer membranes. A homogenate of liver tissue from 6–8-week-old rats was prepared in 0.88 M sucrose. (All sucrose solutions were neutralized with sodium bicarbonate.) It was felt that the hypertonic medium would serve to maintain an intact inner bag during subfractionation of the mitochondria. Tissue (1 g per 9 ml of sucrose) was homogenized at 4° by two strokes with a pestle-type homogenizer (clearance 0.29 mm) at 500 rev./min. The homogenate was strained and centrifuged