THE EFFECT OF Na^+ AND K^+ ON GLYCOLYSIS IN RECONSTITUTED HEMOLYSATES

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1. Introduction

Human erythrocytes maintain high potassium and low sodium ion concentration through the effect of an energy dependent cation pump, counteracting the leakage of the cations across the erythrocyte membrane [1]. Pump activity and glycolysis show a mutual inter dependence. Glycolysis is required for the ATPase pump activity, which, in turn, stimulates glycolysis by providing ADP and inorganic phosphate [2–5]. This mechanism does not exclude the existence of an additional, yet a direct, cation effect on glycolysis.

The present communication examines such a direct effect, taking advanatge of the opposite Na^+ and K^+ content in human and dog erythrocytes: 136 and 19 meq/l of K^+ and Na^+ in the human, in contrast to 10 and 135 for K^+ and Na^+ in the dog erythrocyte [6]. We demonstrate that glycolysis in reconstituted human hemolysate is markedly stimulated by K^+ while the Na^+ counteracts this effect. In contrast, the dog glycolytic system does not show a response to Na^+ and K^+. It is postulated that these patterns reflect an evolutionary adaptation of glycolytic enzymes to the specific erythrocyte ion composition.

2. Materials and methods

2.1. *Preparation of cell free hemolysate*

Human and dog venous blood was obtained in heparin. All further manipulations were conducted at 4°C. The cells were washed three times with 155 mM NaCl solution and the top layer and the buffy coat were discarded. The washed cells were hemolysed by diluting a volume of the packed cells with an equal volume of distilled water. The ghosts were removed by centrifugation at 27 000 g for 20 min to obtain the 'cell free hemolysate'.

2.2. *Gel filtration of the hemolysate*

A column of Sephadex G-25 (fine) measuring 30 × 1 cm, was equilibrated with 50 mM Tris–HCl, pH 7.8 and 1 mM glutathione. Cell-free hemolysate (10 ml) was passed through the column at a rate of 2 ml per min, and, following the void volume, a fraction of 12 ml was collected. Several columns were run in parallel to obtain sufficient gel-filtered hemolysate. This hemolysate contained no detectable ATP, while Na^+ and K^+ were below 2 mM.

2.3. *Measurement of glycolytic activity*

Hemolysates (3 ml) were incubated at 37°C for 1 hr in a final volume of 3.5 ml containing 2 mM glucose, 0.6 mM ATP, 0.6 ADP, 0.15 mM NAD, 0.15 mM NADH, 0.6 mM NADP, 1.2 mM $MgCl_2$ and 3 mM phosphate buffer (either Na^+ or K^+) pH 7.8. Where indicated, NaCl and/or KCl were added.

Glucose was determined by glucose oxide method [7] and lactate by lactic dehydrogenase–NAD method [8].

2.4. *Materials*

Sephadex G-25 was obtained from Pharmacia Fine Chemicals, Uppsala. All chemicals were purchased from Sigma Chemical Co., St. Louis.

3. Results and discussion

3.1. *Reconstitution of glycolysis in gel filtered hemolysate*

Available procedures for modifying erythrocyte cation composition involve prolonged incubation periods or the use of inhibitors [3]. Both were neither desirable nor sufficiently effective for the present study. To obtain a well defined system. in which the ion composition could be easily and accurately controlled, the cations were first removed by gel filtration. No glycolytic activity was demonstrated in gel-filtered hemolysates, unless reconstituted with co-factors, as described in section 2.3.

Higher glucose consumption, yet lower lactate formation was observed in the reconstituted gel filtered system as compared to cell free hemolysate (table 1).

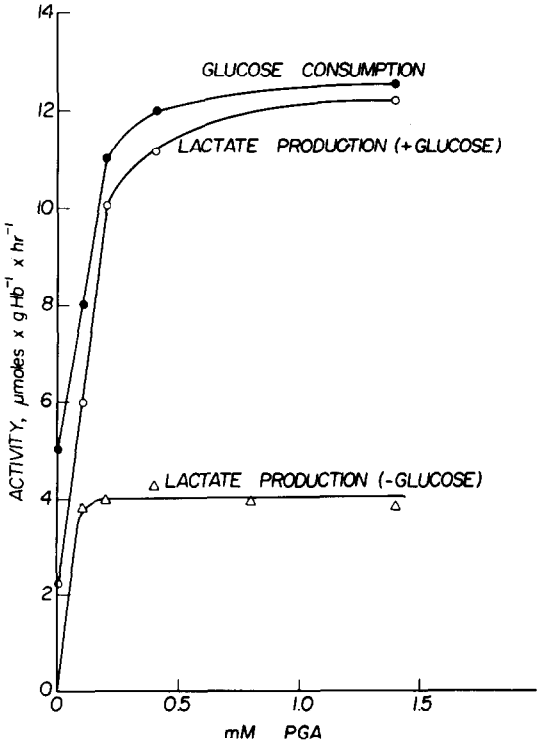

Fig. 1. Glycolytic activity of gel-filtered human hemolysate as affected by PGA. The reaction mixture was as described in section 2.3 but with varying PGA concentration and with the addition of 150 mM KCl. For the low PGA concentration (0.1–0.2 mM), the incubation was restricted to 30 min.

Table 1
Glycolytic activities in human hemolysate.

	Glycolytic activity, μmole × g Hb^{-1} (±S.E.)		
	Glucose consumption (G)	Lactate production (L)	Ratio L/G
Cell-free hemolysate	8.1 ± 0.7	14.2 ± 1.1	1.75
Gel-filtered hemolysate	11.7 ± 0.4	11.0 ± 1.1	1.0

Conditions as described in section 2.3, except that the reaction mixture of the gel-filtered hemolysate was augmented with 150 mM KCl and 0.2 mM PGA.

Noteworthy is the dependence of the reconstituted glycolysis on the addition of 3-phosphoglyceric acid (PGA). Fig. 1 shows that the increased lactate production due to added PGA exceeds the value expected on stoichiometric basis. Furthermore, since glucose consumption is also markedly promoted by PGA, PGA apparently replenishes depleted pools of intermediates. Subsequently, PGA was added at the saturating concentration of 0.2 mM.

3.3. *Ion composition and glycolysis*

The effect of Na^+ and K^+ was studied either individually or in combination. Since the sum of Na^+ and K^+ concentration in both human and dog erythrocytes approximates 150 mM [6], their total concentration was limited to this value. Fig. 2 shows that glycolytic activity of human hemolysate was dependent upon K^+ concentration. Na^+ apparently counteracts the stimulatory effects of K^+, since in the presence of Na^+ higher concentrations of K^+ are required for comparable stimulation.

In dog hemolysates a profoundly different response to Na^+ and K^+ was observed (fig. 3). While lactate production is stimulated by either Na^+ or K^+, the maximal rate was not affected by the sum of (K^+ + Na^+) at all combinations studied. Moreover, glucose utilization of the canine system is entirely independent of ion concentration.

It is of interest that dog or cat erythrocyte ATPase is also unaffected by Na^+ and K^+, while human or rat

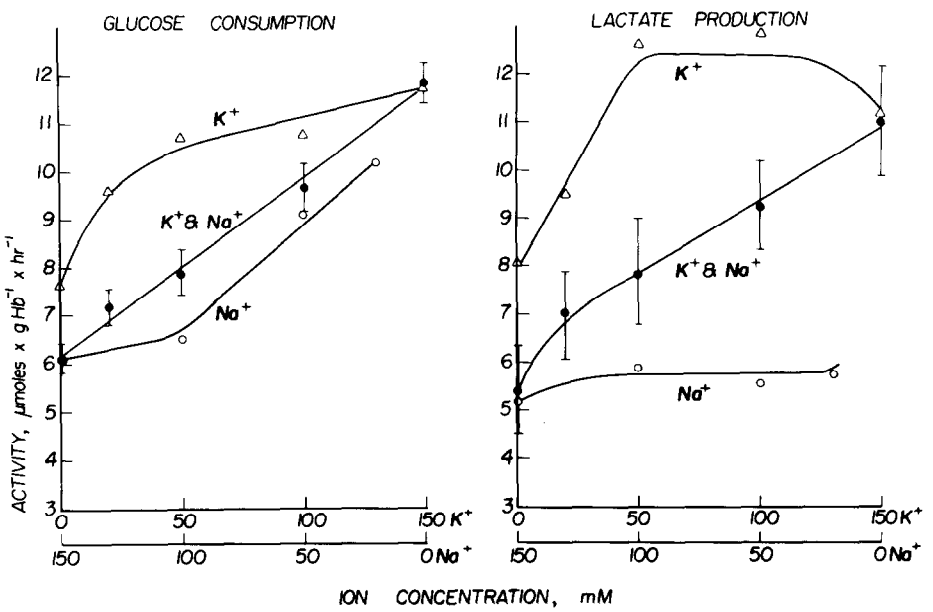

Fig. 2. Glycolytic activity of gel-filtered human hemolysate as affected by Na^+, K^+ and by ($Na^+ + K^+$). Values of standard error for the effect of ($Na^+ + K^+$) are given by horizontal bars.

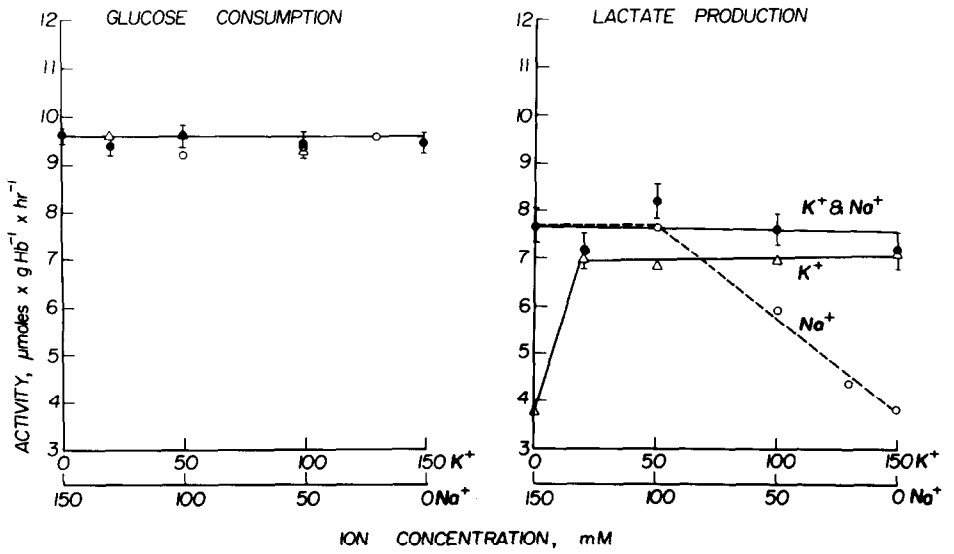

Fig. 3. Glycolytic activity of gel-filtered dog hemolysate as affected by Na^+, K^+ and by ($Na^+ + K^+$). Values of standard error for the effect of ($Na^+ + K^+$) are given by horizontal bars.

erythrocyte ATPase is markedly cation dependent [9]. In a given species, a correlation thus seems to exist between a gradient in cation concentration across the erythrocyte membrane and cation effects on erythrocyte glycolysis and ATPase activity: little or no effect is exerted when the ion composition of the erythrocytes and the plasma is similar. It can therefore be postulated that the response of the erythrocyte glycolysis to Na^+ and K^+ reflects an evolutionary adaptation of one or more of the glycolytic enzymes to the specific erythrocyte ion composition.

Which of the rate limiting glycolytic enzymes are particularly sensitive to a change in erythrocyte composition is not known. Pyruvic acid kinase is a possible candidate, since its activity in extracts of muscle and nerve is stimulated by K^+ and inhibited by Na^+ [10, 11].

In the intact human red blood cell, elevated intracellular Na^+ and extracellular K^+ stimulate glycolysis by activating the ATPase pump and providing ADP [3]. In contrast, our data show a direct, yet an opposite effect of the monocations on glycolysis. These counteracting effects may form a basis for a fine physiological control of glycolytic rates by ion composition.

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