Metabolic properties of low ATP erythrocytes of the monotremes

Hyun Dju Kim*, Robert B. Zeidler, John Sallis[+], Stewart Nicol[†] and Russel E. Isaacks[°]

Department of Pharmacology, University of Alabama in Birmingham, Birmingham, AL, USA, [+] Department of Biochemistry and [†] Department of Physiology, University of Tasmania, Hobart, Tasmania, Australia and *[°] Veterans Administration Medical Center and Department of Medicine, University of Miami, Miami, FL, USA*

Received 16 December 1983

The erythrocytes of the monotremes, having a trace amount of ATP, can metabolize glucose to lactate at a rate comparable to human and other mammalian erythrocytes. The echidna energy metabolism is unique in that adenosine can stimulate glycolytic carbon flow, resulting in a nearly 20-fold net synthesis of ATP.

ATP synthesis *Monotreme erythrocyte* *Glycolysis*

1. INTRODUCTION

We have recently shown that the erythrocytes of the echidna have an extremely low ATP content corresponding only to 2–3% of the high energy phosphate pool seen in human erythrocytes [1]. The platypus, the other sole surviving monotreme, has also been found to possess erythrocytes exhibiting a similar trait [2]. In neither species could significant amounts of energy-rich purine or pyrimidine compounds be detected. However, the monotreme erythrocytes have an abundance of 2,3-bisphosphoglycerate and other phosphorylated compounds [1,2]. The requirement for free energy to meet the need of such cellular functions as active cation transport was suggested by the observation on the ionic distribution across the cell membranes. In the echidna, cellular K content is several times greater and cell Na is much less than the plasma levels [1,3]. Although the generation of free energy would be expected to originate from glycolysis, the extent to which the blood-borne glucose is consumed by these cells is, to date, unknown. The aim of this communication is to determine whether the glycolytic pathway can be energized by this low level of ATP in the erythrocytes of the monotremes.

We found that, of possible substrates including dihydroxyacetone, ribose, glucose, inosine and adenosine, glucose is by far the most preferred by erythrocytes of both species. Lactate production from glucose amounted to 2.66 ± 0.27 μmol · ml cells^{-1} · h^{-1} in the platypus and 3.26 ± 0.46 μmol · ml cells^{-1} · h^{-1} in the echidna erythrocytes. Ribose is not used. Erythrocytes of the echidna can consume dihydroxyacetone to a much lesser extent. Of particular interest is the finding that although adenosine by itself is a poor substrate, adenosine together with glucose augmented the ATP content nearly 20-fold in the echidna cells. These findings imply that the low cellular ATP content of 0.03 μmol/ml cells is apparently sufficient to energize the glycolytic pathways.

2. MATERIALS AND METHODS

Four echidna and four platypuses were captured in Tasmania. The animals were anesthetized by

* New address: Department of Pharmacology, University of Missouri-Columbia, School of Medicine, Columbia, MO 65212, USA

Published by Elsevier Science Publishers B.V.
001457593/84/$3.00

halothane, and blood samples (6–10 ml) were taken from each animal by cardiac puncture using EDTA as an anticoagulant. The cells were washed several times with cold 0.15 M NaCl and the white buffy coat was removed after each centrifugation. The washed cells were resuspended to give a hematocrit of approximately 15% at 37°C in a balanced salt medium composed of 120 mM Na, 5 mM K, 20 mM phosphate buffer at pH 7.4 and 5 mM metabolic substrates. Where ribose was used, its concentration was 3 mM in view of a substrate inhibition of metabolism seen in other cells when higher concentrations were used [4,5]. At frequent intervals, aliquots of cell suspensions were taken and immediately extracted with 0.56 M perchloric acid (PCA) and neutralized with K_2CO_3 as in [4,5].

The PCA extracts were frozen and transported on dry ice to the University of Alabama in Birmingham. Glucose was assayed by glucose oxidase and peroxidase coupled with *o*-dianisidine as chromagen (Sigma kit no.510). Lactate was determined as in [6]. Phosphorylated compounds were determined by the ion-exchange column chromatography procedure in [7,8] using equal portions of DA-X4-20 (Durrum) and Aminex A-25 (Bio-Rad) resins as in [9]. The size of the precolumn was 3 × 150 mm and the main column was 3 × 500 mm. Both were eluted with a linear gradient from 0.1 to 0.6 M ammonium chloride containing 0.5 M potassium tetraborate. The column was eluted with a volume of 160 ml within 6 h. The UV absorption and inorganic phosphate content resulting from hydrolysis of organic compounds by heat combustion were continuously and automatically monitored. Inorganic phosphate (P_i) was assayed colorimetrically after simultaneous addition of sulfuric acid containing ammonium molybdate and ascorbic acid. The identification of phosphorylated compounds was determined from elution positions corresponding to known standards. ATP was measured by luciferin-luciferase reaction on a luminometer (LKB Model 1250).

3. RESULTS

The rates of glucose consumption and concomitant lactate production from erythrocytes of the monotremes suspended in a balanced salt solution fortified with glucose are summarized in table 1. It is evident that the seemingly inadequate ATP content does not hamper the ability of these cells to use glucose.

Table 1

Glycolytic capacity of monotreme erythrocytes

Species	Glucose consumption[a] (± SE)	Lactate production (μmol · ml cell^{-1} · h^{-1}) (± SE)	Ratio $\frac{\text{Lactate}}{\text{Glucose}}$
Platypus	1.71 ± 0.15 (n = 3)	2.66 ± 0.27 (n = 4)	1.55 ± 0.31
Echidna	1.83 ± 0.26 (n = 4)	3.26 ± 0.46 (n = 4)	1.82 ± 0.45

[a]Taken from [12]

One of the 4 platypus samples exhibited glucose consumption rate of 4.20 μmol · ml cell^{-1} · h^{-1} without correspondingly high lactate production. Accordingly, this figure is not included in table 1. n = number of determinations. Experimental conditions: P_i (20 mM), pH 7.4, HcT (~ 15%), 37°C. The plasma glucose level of the echidna and the platypus were 114 mg/dl and 74 mg/dl, respectively

Fig. 1 depicts the lactate production from the platypus cells suspended in a variety of metabolic substrates. The erythrocytes of the platypus failed to consume dihydroxyacetone, ribose or inosine.

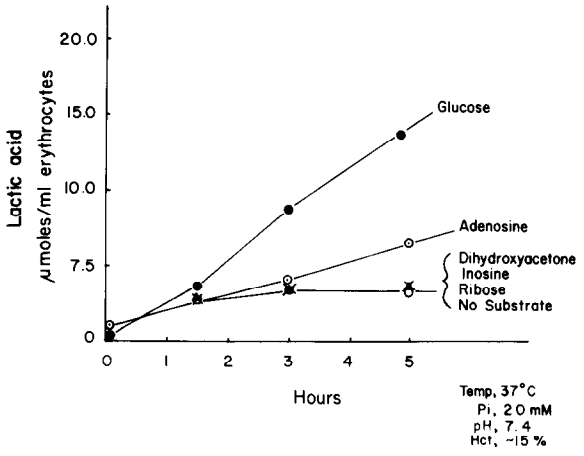

Fig. 1. Lactic acid production in erythrocytes from the duckbill platypus suspended in a variety of metabolic substrates.

However, a small amount of the lactate was produced from adenosine.

Glucose is again the metabolic substrate preferred by the echidna cells as shown in fig. 2a. Lactate production by dihydroxyacetone ranges from 1.50 to 1.75 μmol · ml cells · h^{-1} for the first 2 h of incubation, followed by a much slower rate thereafter. Of particular interest is the finding regarding adenosine metabolism. Although adenosine by itself was poorly metabolized to form lactate, the rate of lactate production in cells treated together with glucose and adenosine was much greater than the sum of lactate produced by either adenosine or glucose alone. These findings suggest that adenosine directly stimulated glycolysis in the echidna cells akin to the activation of glycolysis seen in the cow erythrocytes by purine compounds [10,11].

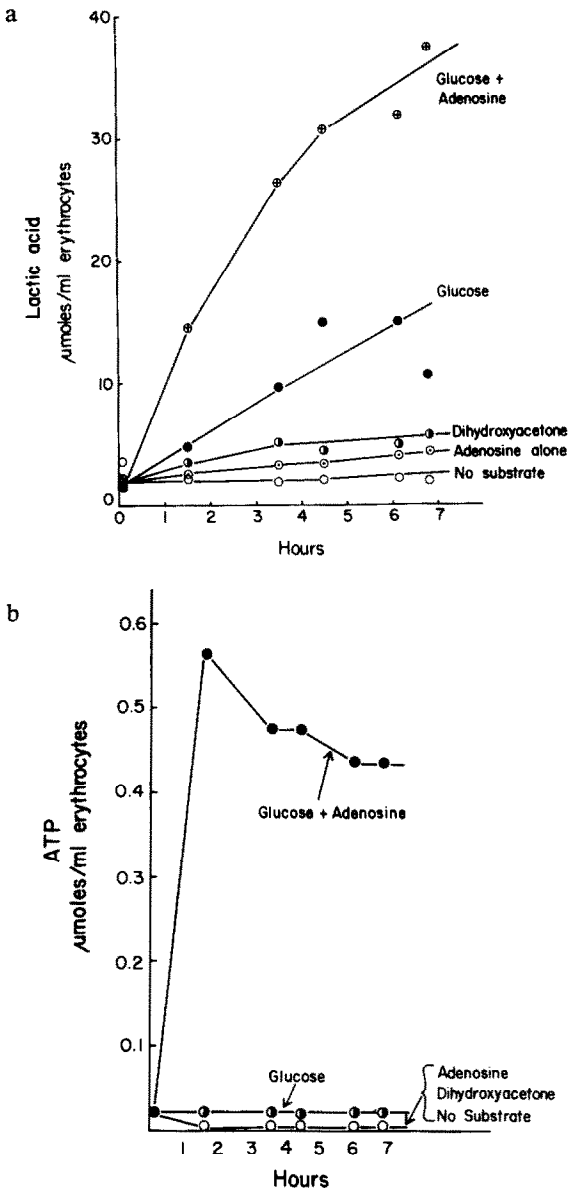

Fig. 2 (a). Metabolic response of erythrocytes from the short-beaked echidna to a variety of substrates. The experimental conditions are the same as in fig. 1 (b). ATP content of the echidna cells suspended in various substrates.

In fig. 2b, the changes in ATP levels are presented. In keeping with the findings in [1,2], the echidna cells have a low ATP content of 0.02 μmol/ml cells. As in other mammalian cells, glucose maintained this low cellular ATP during in vitro incubation for nearly 7 h at 37°C. When the cells were deprived of glucose, ATP fell rapidly to an unmeasurable level. As expected, neither adenosine nor dihydroxyacetone sustained the cellular ATP. However, a spectacular finding is the 20-fold increase in the net synthesis of ATP concomitant with enormous lactate production by the echidna cells suspended in glucose plus adenosine medium.

In order to corroborate this finding, the PCA extracts of adenosine-treated cells in fig. 2 were combined and subjected to ion-exchange column chromatography as shown in fig. 3. It should be noted that the large P_i peak which appears reflects the presence of extracellular P_i used as a buffer. Because of the size of P_i, it is difficult to quantitate hexose-6-P which elutes in the descending shoulder of the P_i peak. The presence of 2,3-DPG, amounting to 4.5 μmol/ml cells, is clearly evident. The elution positions shown in fig. 3 corresponding to ADP and ATP were devoid of both phosphate and any UV absorption at 254 nm, implying that the PCA extract derived from 67 μl packed cells was too small to allow detection of the low nucleotide concentrations. Fig. 4 shows an ion-exchange chromatogram of the erythrocytes of the echidna treated together with adenosine and glucose. For the purpose of comparison, the PCA extracts from the same number of erythrocytes as glucose-exposed cells were subjected to ion-exchange column chromatography. The appearance of a large ATP peak is evident. In both columns, two compounds, whose identities are unknown, were eluted

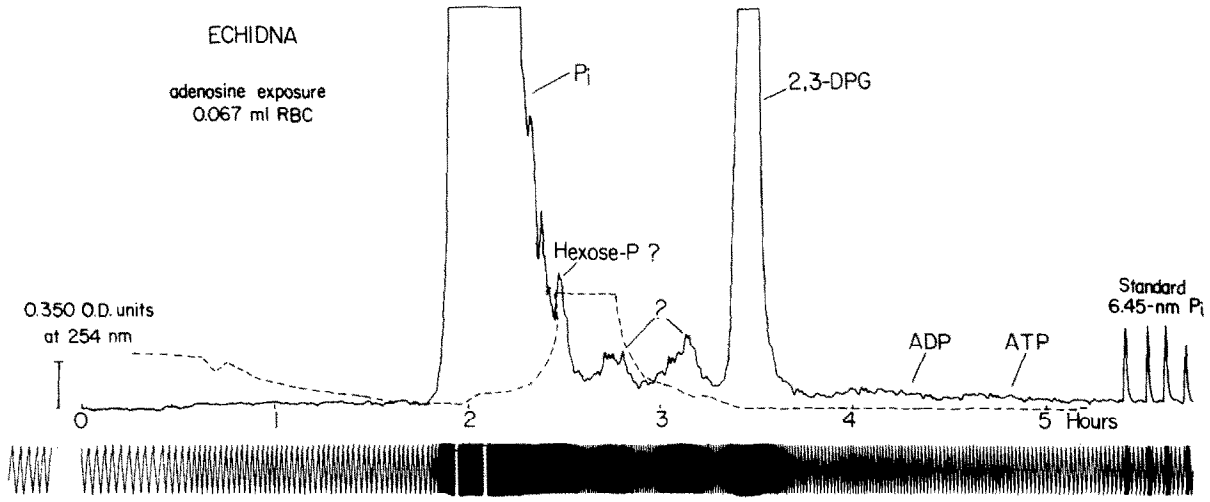

Fig. 3. Ion-exchange column chromatography of phosphorylated compounds in echidna cells suspended in adenosine. The solid line depicts the phosphate recording, whereas the dotted line represents the absorbance measurement at 254 nm.

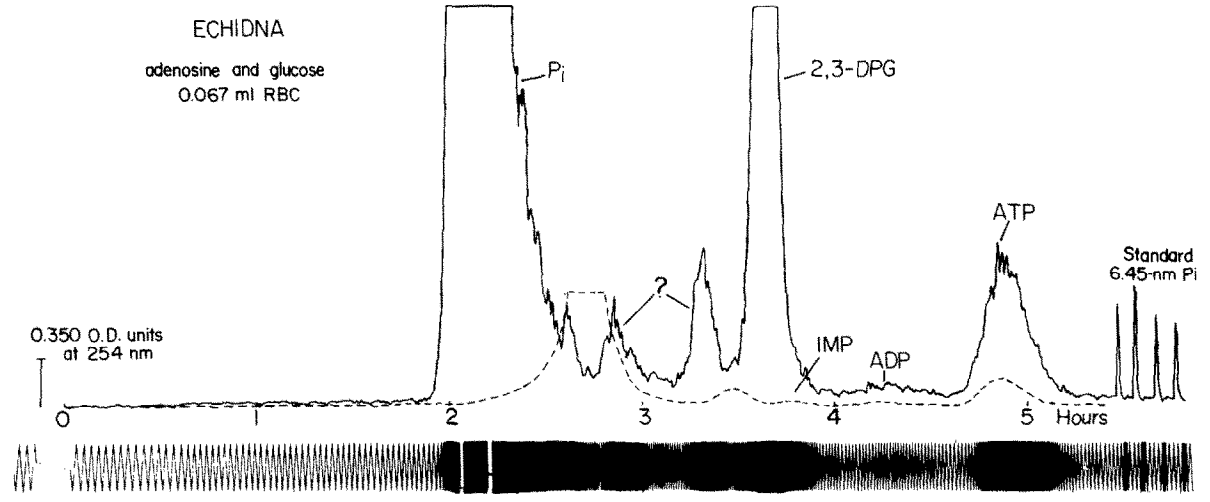

Fig. 4. Ion-exchange column chromatography of phosphorylated compounds from echidna cells exposed to adenosine and glucose.

prior to 2,3-DPG. These compounds were present even in freshly drawn echidna cells [1].

4. DISCUSSION

Glycolytic rates of mammalian erythrocytes vary over a wide range [12]. The adult pig red cell is almost totally devoid of glucose metabolism [4,5], whereas the rat erythrocyte is a prolific glucose user. The results reported herein clearly show that the monotreme erythrocytes are similar to other mammalian cells, preferring glucose as the major energy source. However, the extent to which glucose can be consumed by the monotreme cells is surprising in view of the low ATP content of 0.03 μmol/ml packed cells. At this ATP concentration, human cell glycolytic carbon flow would be grossly curtailed, since hexokinase activity with a K_m for

ATP of 0.6 μmol/ml cells [13] would be largely inactivated.

The net synthesis of ATP in the presence of adenosine supplemented with glucose occurs in a number of species including sheep [14], horse [14], calf and cow [15]. Although adenosine alone is a poor substrate in every case, net ATP formation takes place when an alternate carbon source is available. In stimulating ATP synthesis, glucose can be replaced by inosine in the inosine permeable sheep erythrocytes [14], and by dihydroxyacetone and glyceraldehyde in calf cells [15]. The factors controlling the metabolic pathways for this phenomenon have been extensively discussed in [14]. Like the erythrocytes of the horse, sheep and cow, the erythrocytes of the echidna possess the capacity to modulate their ATP pool size. However, what distinguishes the echidna cells from the erythrocytes of the other species is their enormous reserve of metabolic ability to augment cellular ATP as evidenced by a nearly 20-fold increase in the presence of adenosine and glucose. An equally fascinating observation is the production of large amounts of lactate concomitant with the increased ATP pool. It is unlikely that the ribose moiety of the nucleoside is the carbon source for lactate, since neither adenosine nor inosine is readily metabolized by the echidna erythrocytes. Rather, the augmented lactate is most likely accounted for by increased glucose consumption activated by nucleoside in much the same manner as that which occurs in the cow red cells [10,11]. It will be interesting to characterize the monotreme glycolytic enzymes which apparently can carry out hexose phosphorylation with a trace amount of ATP.

ACKNOWLEDGEMENTS

The expert technical assistance of Martha Baird and Betty Kubas is gratefully acknowledged. Also, we would like to thank Genie Eckenfels for typing the manuscript. This work is supported, by NIH grant no. AM 20815 (R.E-I.), AM 27570 (H.D.K.) and by an NSF grant no. PCM 7815821. Special thanks are due to the National Parks and Wildlife Service of Tasmania for permission to work on the monotremes.

REFERENCES

[1] Kim, H.D., Zeidler, R.B., Sallis, J.D., Nicol, S.C. and Isaacks, R.E. (1981) Science 213, 1517–1519.
[2] Isaacks, R.E., Nicol, S.C., Sallis, J.D., Zeidler, R.B. and Kim, H.D. (1983) Am. J. Physiol., in press.
[3] Kim, H.D., Baird, M., Sallis, J., Nicol, S. and Isaacks, R.E. (1983) submitted.
[4] Kim, H.D. and McManus, T.J. (1971) Biochim. Biophys. Acta 230, 1–11.
[5] Kim, H.D. and McManus, T.J. (1971) Biochim. Biophys. Acta 230, 12–19.
[6] Lundholm, L., Mohm-Lundholm, E. and Vamos, N. (1963) Acta Physiol. Scand. 58, 243–249.
[7] Bessman, S.P. (1974) Anal. Biochem 59, 524–532.
[8] Bessman, S.P., Geiger, P.J., Luand, T. and McCabe, E.R. (1974) Anal. Biochem. 59, 533–546.
[9] Zeidler, R.B. and Kim, H.D. (1982) J. Cell Physiol. 112, 360–366.
[10] Seider, M.J. and Kim, H.D. (1979) Am. J. Physiol. 236, C255–C261.
[11] Seider, M.J. and Kim, H.D. (1979) Am. J. Physiol. 236, C262–C267.
[12] Kim, H.D. (1983) in: Red Blood Cell in Domestic Mammals (Agar, N.S. and Board, P.G. eds) pp. 339–355, Elsevier, Amsterdam, New York.
[13] Stocchi, V., Magnani, M., Canestrari, F., Dacha, M. and Fornaini, G. (1982) J. Biol. Chem. 257, 2357–2364.
[14] McManus, T.J. (1973) in: The Human Red Cell in vitro (Greenwalt, T.J. and Jamieson, G.A. eds) pp. 49–64.
[15] Kim, H.D. (1979) Biochim. Biophys. Acta 588, 44–54.