Volume 258, number 1, 143–146 FEB 07859 November 1989

Effect of adenosine on fructose 2,6-bisphosphate levels and glucose metabolization by chicken erythrocytes

Carme Espinet, Ramon Bartrons and José Carreras

Departament de Ciéncies Fisiològiques, Humanes i de la Nutrició, Unitat de Bioquímica, Facultat de Medicina, Universitat de Barcelona, Zona Universitària de Pedralbes, 08028 Barcelona, Spain

Received 6 October 1989

Adenosine can be metabolized by chicken erythrocytes as a carbon source maintaining ATP levels. In addition, it stimulates glucose uptake and lactate production. Adenosine effects do not involve binding to membrane receptors. They are dependent on the provision of a carbon source to glycolysis and correlate with the increase of fructose 2,6-bisphosphate levels that activating phosphofructokinase would increase the glycolytic flux.

Adenosine; Fructose 2,6-bisphosphate; Erythrocyte; (Chicken)

1. INTRODUCTION

Although the ability to consume glucose by erythrocytes varies greatly, depending upon species and cell and animal age, most mammalian erythrocytes use glucose as the major metabolic fuel. Exceptions are pig erythrocytes which are practically impermeable to glucose a few weeks after birth and cow erythrocytes in which glycolytic rate decreases postnatally as a consequence of overall changes in the enzymes and cofactors [1,2]. In contrast to mammalian erythrocytes, avian erythrocytes do not consume measurable glucose under basal conditions [3]. Only traces of $^{14}CO_2$ were produced when [1-^{14}C]glucose was added to chicken, goose and duck erythrocytes [4], and no detectable amounts of lactate were produced when fowl erythrocytes were incubated with glucose [5,6]. It has been found that adenine nucleosides constitute alternate metabolic fuel for pig erythrocytes and activate glycolysis in cow erythrocytes [1,2]. This paper reports the effects of adenosine on glucose metabolism and on fructose 2,6-P_2 levels in chicken erythrocytes. Fructose 2,6-P_2 is a very powerful stimulator of chicken erythrocyte phosphofructokinase [7], that begins to increase at hatching and accumulates progressively to reach adult levels in a few days [8].

2. MATERIALS AND METHODS

[U-^{14}C]Glucose and 2-[1-^{14}C]deoxy-D-glucose were purchased from Nuclear Iberica. Dipiridamol was from Acofarma, and deoxycoformycin, α,β-methylene ADP and L-N_6-phenylisopropyl adenosine (L-PIA) were from Sigma. Enzymes and other biochemical reagents were from either Boehringer or Sigma. Dowex AG-1-X8 and Dowex 50 WX8 were from Bio-Rad.

White Leghorn adult chickens were obtained from a poultry farm. Blood samples were collected from the jugular vein and drawn into 1 vol. ice-cold 150 mM NaCl, containing 15 mM sodium citrate and 5 mM glucose, pH 7.2. Red blood cells were quickly washed 3 times with the same medium without citrate at 0–3°C and resuspended, unless otherwise indicated as a 20% hematocrit, in a medium containing: 4.2 mM imidazol, 7.4 mM glycylglycine, 137 mM NaCl, 5.9 mM KCl, 2.4 mM $MgSO_4$, 1.2 mM KH_2PO_4, and 1.25 mM $CaCl_2$, pH 7.4, gassed with 95% O_2/15% CO_2. Incubations were performed at 40°C in a shaking water bath gassing each 10 min. Substrates were added after preincubation in the presence or in the absence of inhibitors during 20 min. Incubation was stopped either by freezing in an acetone/carbonic bath or by the addition of perchloric acid at a 5% final concentration.

Phosphofructo 2-kinase (PFK-2) activity was assayed as in [9]. Fru 2,6-P_2 was determined in NaOH extracts as in [10]. Glucose 6-P/fructose 6-P, ATP and lactate were assayed in perchloric neutralized extracts essentially as reported by Beutler [11]. $^{14}CO_2$ production from [U-^{14}C]glucose was assayed as radioactivity trapped in hyamide hydroxide [12]. The rate of glucose uptake into the glycolytic flux was determined as the conversion of [U-^{14}C]glucose in phosphorylated or charged intermediates [13]. [U-^{14}C]Glucose was separated from the ^{14}C-labeled glycolytic intermediates by Dowex ion exchange chromatography [14]. After elution of glucose with water, glycolytic intermediates were eluted with a 0.1–1 N gradient of formic acid.

The transport of 2-[1-^{14}C]deoxy-D-glucose was determined essentially as described by Simons et al. [15]. At different times of incubation, samples were centrifuged in a microfuge at 12000 × g for 3 min, and the cells were washed 3 times with the incubation medium without addition of substrate. Liquid scintillation counting was performed in perchloric extracts of washed cells to avoid haemoglobin quenching.

3. RESULTS AND DISCUSSION

As summarized in table 1, when chicken erythrocytes were incubated in the absence of metabolic substrates,

Correspondence address: C. Espinet, Departament de Ciéncies Fisiològiques, Humanes i de la Nutrició, Unitat de Bioquímica, Facultat de Medicina, Universitat de Barcelona, Zona Universitària de Pedralbes, 08028 Barcelona, Spain

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

Table 1

Effect of substrates on ATP, lactate, hexose phosphates and fructose 2,6-P_2 levels in chicken erythrocytes

Addition	ATP	Lactate	Hexoses 6-P	Fru 2,6-P_2
None	46.7 ± 5.2	104.2 ± 5.8	83.5 ± 11.6	28.0 ± 15.0
Glucose	64.5 ± 6.0	107.5 ± 6.5	208.0 ± 23.0	174.5 ± 18.6
Adenosine	85.0 ± 5.0	400.0 ± 11.6	255.0 ± 16.0	246.0 ± 34.0
Glucose + adenosine	99.0 ± 1.0	519.5 ± 15.6	350.0 ± 16.0	580.0 ± 0.001
Inosine	105.7 ± 0.25	280.0 ± 17.2	705.0 ± 9.6	796.5 ± 37.0
Glucose + inosine	103.0 ± 2.3	486.0 ± 23.7	1032.0 ± 25.6	1683.0 ± 43.0

Erythrocytes were incubated for 4 h at 40°C in the presence of 5 mM of the indicated substrates. The values correspond to the % ± SE of initial levels in two different experiments. The initial contents (nmol/10^9 cells) were: 144 ± 24; lactate, 29 ± 0.2; hexose phosphates, 2.8 ± 0.45; fructose 2,6-P_2, 0.15 ± 0.02

ATP declined during 4 h of incubation to about 50% of the initial value (144.25 ± 24 nmol/10^9 cells). The decline in ATP content was less steep in the presence of either glucose or adenosine, alone or in combination. Lactate levels did not vary in chicken erythrocytes incubated either in the absence of substrates or with glucose, but lactate was produced when the cells were suspended with either adenosine alone or in combination with glucose (fig.1A, table 1). The inability of glucose to maintain ATP levels and to produce lactate accumulation confirms the low glucose consumption by chicken erythrocytes [3,5,6]. The ability of adenosine to be utilized as a carbon source for lactate formation indicates that chicken erythrocytes possess an enzyme system for the conversion of adenosine to inosine and for the cleavage of inosine to hypoxanthine and ribose 1-P, which in turn would provide glycolytic intermediates [16,17]. The failure to accumulate ATP when suspended in the nucleoside suggests that chicken erythrocytes, similarly to some mammalian erythrocytes [2,17–19], do not possess a very effective enzyme system for adenosine metabolization.

Lactate production by chicken erythrocytes suspended in glucose plus adenosine was greater than the sum of lactate production from glucose and adenosine alone (table 1, fig.1A), and the rate of glucose conversion into phosphorylated or charged intermediates was found to be increased about 1.4-fold by adenosine (fig.1C). These results indicate that adenosine might have some synergistic effect with glucose on either the rate of glycolysis or the rate of glucose uptake into the erythrocytes. It has been reported that human erythrocyte membrane carriers for hexoses and nucleosides have several structural features in common, and that hexose transport is inhibited by adenosine derivatives in human erythrocytes [20]. Results in fig.1B show that 2-deoxy-D-[^{14}C]glucose entry into chicken erythrocytes was not influenced by adenosine, suggesting that activation by adenosine of erythrocyte lactate production was due to stimulation of glycolysis.

It has been found that inositol pentaphosphate (IP_5), which is present at mM concentration in adult avian erythrocytes, is a powerful inhibitor of chicken erythrocyte phosphofructokinase [6] and we have recently reported that Fru-2,6-P_2 can counteract IP_5 inhibition [7]. Therefore, the possibility that activation of chicken erythrocyte glycolysis by adenosine was mediated by Fru-2,6-P_2 was considered. As shown in table 1 and fig.1E, in chicken erythrocytes incubated in the absence of metabolic substrates Fru-2,6-P_2 fell to very low levels. Incubation with glucose or adenosine alone produced a progressive accumulation of Fru-2,6-P_2, which was higher when the erythrocytes were suspended in glucose plus adenosine.

The action of adenosine on Fru-2,6-P_2 levels could result from either an increase in the levels of PFK-2 substrates and/or effectors or an activation of PFK-2 secondary to the binding of adenosine to a membrane receptor. It has been suggested that purine compounds stimulate glycolysis in cow erythrocytes by probably binding to membrane receptors [2,18] and it has been reported that adenosine decreases the concentration of Fru-2,6-P_2 in hepatocytes through the stimulation of adenylate cyclase secondary to the binding of adenosine to membrane receptors [21]. The inability of the adenosine analog L-PIA to produce lactate accumulation (fig.1A) and to increase erythrocyte Fru-2,6-P_2 (fig.1E) seems to rule out the involvement of adenosine binding to a membrane receptor in the mechanism of these effects. In agreement with this, PFK-2 activity (0.25 mU/10^9 cells) did not vary when chicken erythrocytes were incubated with either glucose or adenosine (not shown).

As shown in fig.1E, the inhibitor of adenosine transport dipiridamol impeded the increase of erythrocyte Fru-2,6-P_2 concentration produced by adenosine, suggesting that this effect was dependent upon the entry of adenosine into the red cells. Furthermore, as summarised in table 2, in the presence of the adenosine deaminase inhibitor deoxycoformycin and

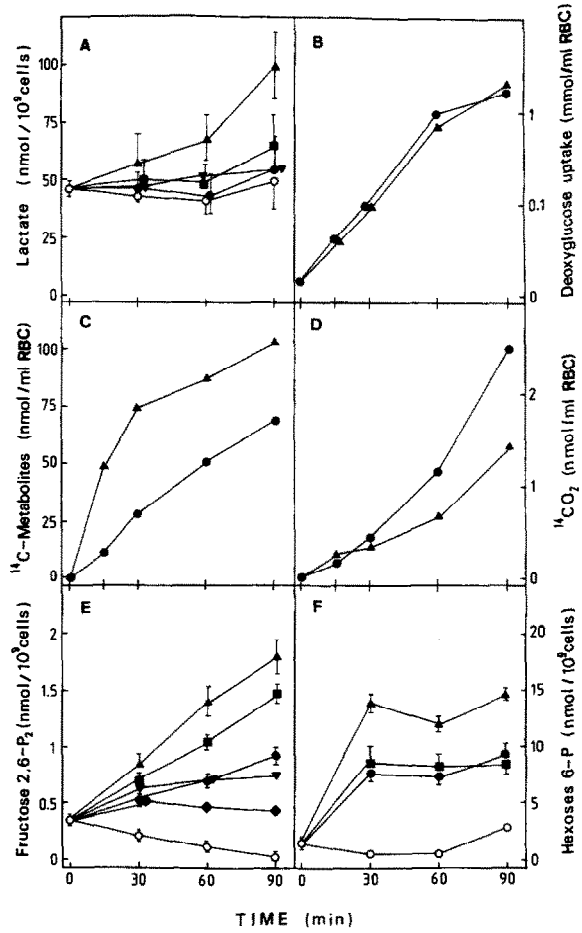

Fig.1. Time course of the effect of substrates on lactate accumulation (A), 2-[1-^{14}C]deoxy D-glucose uptake (B), conversion of [U-^{14}C]glucose into phosphorylated or charged metabolites (C), $^{14}CO_2$ production (D), Fru 2,6-P_2 levels (E) and hexose phosphate concentration (F). (○) Control; (●) 20 mM glucose; (■) 1 mM adenosine; (▲) 20 mM glucose plus 1 mM adenosine; (▼) 20 mM glucose plus 0.3 mM L-PIA; and (◆) 1 mM adenosine plus 0.8 mM dipiridamol. Bars represent SE for 2–4 experiments.

the 5′-nucleotidase inhibitor, α,β-methylene ADP, the production of Fru-2,6-P_2 and lactate in chicken erythrocytes induced by adenosine was inhibited. This indicates that the two effects are dependent upon the provision of a carbon source to glycolysis. The results in table 1 and fig.1F show that when chicken erythrocytes were suspended in adenosine, hexose phosphate concentration increased, and the results summarized in table 2 demonstrate that the increase was suppressed by the presence of adenosine deaminase and 5′-nucleotidase inhibitors. In contrast, these inhibitors did not modify inosine effects (tables 1 and 2). Incubation of chicken erythrocytes with inosine maintained ATP concentration and increased lactate, hexose phosphates and Fru 2,6-P_2 levels at values even higher than those produced by adenosine. This suggests that in chicken erythrocytes the rate of inosine uptake and metabolization is higher than that of adenosine, although mammalian erythrocytes are more permeable to adenosine than to inosine [17].

Table 2

Effect of the inhibitors of adenosine metabolism on production of lactate, fructose 2,6-P_2 and hexose phosphates in chicken erythrocytes induced by pentose nucleosides

Addition	Lactate	Fru 2,6-P_2	Hexoses 6-P
Adenosine	16.8 ± 1.2	0.75 ± 0.02	4.0 ± 0.1
Adenosine + inhibitors	13.5 ± 0.2	0.30 ± 0.01	1.7 ± 0.07
Inosine	20.0 ± 1.0	0.71 ± 0.02	5.2 ± 0.1
Inosine + inhibitors	22.0 ± 3.9	0.87 ± 0.07	6.4 ± 0.5

Adenosine and inosine were 0.1 mM, deoxycoformycin was 0.2 mM, α,β-methylen ADP 1 mM. The incubation time was 60 min. Values are nmol/10^9 cells ± SD

Results in fig.1D show that $^{14}CO_2$ production from [U-^{14}C]glucose was decreased 50% when chicken erythrocytes were incubated in the presence of adenosine. This effect could result from either $^{14}CO_2$ fixation catalyzed by the ribulose diphosphate carboxylase system [21] or the modification of pyruvate oxidation. The last possibility seems to be discarded since the formation of $^{14}CO_2$ from [^{14}C]pyruvate by chicken erythrocytes (4.3 nmol/min × ml RBC) was not influenced by the presence of 1 mM adenosine (not shown).

In conclusion, it is confirmed that glucose is a poor substrate for chicken erythrocytes. It is shown that adenine nucleosides are alternative substrates and stimulate glucose uptake into the glycolytic flux. Adenosine effects do not involve adenosine binding to membrane receptors and are dependent on the provision of a carbon source to glycolysis. Stimulation of the glycolytic flux correlates with the increase of Fru 2,6-P_2 levels which can counteract phosphofructokinase inhibition [7]. Fru 2,6-P_2 could also be involved in the adenosine effects described in some mammalian erythrocytes [2,17–19].

Acknowledgements: This work has been supported by CAICYT and FIS. C.E. is the recipient of a Fellowship from the Ministerio de Educación y Ciencia, Spain.

REFERENCES

[1] Kim, H.D. (1983) in: Red Blood Cells of Domestic Mammals (Agar, N.S. and Board, P.G. eds) pp.339–355, Elsevier, Amsterdam.
[2] Kim, H.D. (1985) in: Circulation, Respiration, and Metabolism (Gilles, R. ed.) pp.312–321, Springer, Berlin.
[3] Bell, D.J. (1971) in: Physiology and Biochemistry of the Domestic Fowl (Bell, D.J. and Freeman, B.M. eds) vol.2, pp.863–872, Academic Press, London.
[4] Shields, C.E., Herman, Y.F. and Herman, R.H (1964) Nature 203, 935–936.
[5] Bell, D.J. and Culbert, J. (1968) Comp. Biochem. Physiol. 25, 627–637.

[6] Rosa, R., Rosa, C.D., Ocampos, D. and Bacila, M. (1983) Comp. Biochem. Physiol. 75B, 141–145.
[7] Espinet, C., Bartrons, R. and Carreras, J. (1988) Comp. Biochem. Physiol. 90B, 453–457.
[8] Espinet, C., Bartrons, R. and Carreras, J. (1986) FEBS Lett. 209, 254–256.
[9] Bartrons, R., Hue, L., Van Schaftingen, E. and Hers, H.G. (1983) Biochem. J. 214, 829–837.
[10] Van Schaftingen, E., Lederer, B., Bartrons, R. and Carreras, J. (1985) Eur. J. Biochem. 129, 191–195.
[11] Beutler, E. (1975) in: Red Cell Metabolism (Beutler, E. ed.) Grune and Stratton, New York.
[12] McElroy, F.A., Kinlough-Rathbone, R.L., Ardlie, N.G., Packham, M.A. and Mustard, J.E. (1971) Biochim. Biophys. Acta 253, 64–77.
[13] Lucas, M. (1987) Biomed. Biochim. Acta 46, 253–257.
[14] Golden, S., Chenowetz, M., Dunn, A., Okajima, F. and Katz, J. (1981) J. Am. Physiol. 241, 121–128.
[15] Simons, T.J.B. (1983) J. Physiol. 338, 477–499.
[16] Bontemps, F., Van den Berghe, G. and Hers, H.-G. (1986) J. Clin. Invest. 77, 824–830.
[17] McManus, T.J. (1974) in: The Human Red Cell in Vitro (Greenwalt, T.J. and Jarrison, G.A. eds) pp.49–63, Grune and Stratton, New York.
[18] Seider, M.J. and Kim, H.D. (1979) Am. J. Physiol. 236, C255–C261.
[19] Kim, H.D., Zeidler, R.B., Sallis, J., Nicol, S. and Isaaks, R.E. (1984) FEBS Lett. 167, 83–87.
[20] May, J.M. (1988) J. Cell. Physiol. 135, 332–338.
[21] Bartrons, R., Van Schaftingen, E. and Hers, H.-G. (1984) Biochem. J. 218, 157–163.
[22] Lionetti, F.J. (1974) in: Cellular and Molecular Biology of Erythrocytes (Yashikawa, H. and Rapaport, S. eds) pp.143–166, University of Tokyo Press, Tokyo.