Volume 313, number 2, 126–128 FEBS 11775 November 1992

Glucose decreases respiratory control ratio in EL-4 tumor cells

Vladimir L. Gabai

Institute of Medical Radiology, Russian Academy of Medical Sciences, Obninsk 249020, Russian Federation

Received 21 September 1992

EL-4 ascites thymoma cells are shown to have high aerobic glycolysis and decreased Pasteur effect. At the same time, glucose produces a much smaller inhibitory effect on cell respiration (Crabtree effect) than in Ehrlich ascites carcinoma (EAC) cells. In intact EL-4 cells, the respiratory control ratio (RCR) was found to be 6.2 with endogenous substrates and 8.0 with glutamine. Glucose decreased the RCR to 3.2, by stimulating the state 4 respiration. In rat thymocytes and EAC cells, such an effect of glucose was absent (RCR of 7.0 and 7.2, respectively). It is suggested that in EL-4 tumor cells, the high aerobic glycolysis and small Crabtree effect may be due to glucose-induced 'uncoupling' of oxidation and phosphorylation.

Respiration; Aerobic glycolysis; Respiratory control; Oxidative phosphorylation; EL-4 ascites tumor cell

1. INTRODUCTION

Rapidly growing tumor cells have some unusual bioenergetic features: increased aerobic glycolysis, Crabtree effect (inhibition of respiration by glucose) and decreased Pasteur effect [1,2]. Several mechanisms for such energy metabolism have been suggested, e.g. small mitochondria content and their abnormal structure [1,2], elevated ATPases activities [3,4], high hexokinase activity and its association with mitochondrial outer membrane [5], increased level of fructose-2,6-diphosphate, a glycolysis activator [6]. However, the contribution of the proposed mechanisms in the bioenergetics of tumor cells has not been fully evaluated.

The inhibitory effect of glucose on tumor cell respiration has been intensively studied [7–9]. The most accepted explanation of this effect is the limited access of ADP and phosphate for H^+-ATP-synthase of mitochondria in the presence of glucose, although another mechanism (decreased respiratory substrates availability) was also suggested [10]. The effect of glucose on the respiratory control ratio (RCR) has not been studied systematically and there have only been a few reports about the RCR of intact tumor cells (e.g. in AS-30D hepatoma the RCR was found to be equal to 6–7 [11]).

Below a new mechanism of high aerobic glycolysis in tumor cells is described, i.e. glucose-induced decrease in efficiency of oxidative phosphorylation.

Correspondence address: V.L. Gabai, Institute of Medical Radiology, Russian Academy of Medical Sciences, Korolev's 4, Obninsk 249020, Russian Federation. Fax: (7) (095) 255-2486.

Abbreviations. CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DNP, 2,4-dinitrophenol, EAC, Ehrlich ascites carcinoma; HBSS, Hank's balanced salt solution; RCR, respiratory control ratio.

2. MATERIALS AND METHODS

The EL-4 ascites thymoma and Ehrlich ascites carcinoma (EAC) were grown as described previously [12]. The cells were washed with HBSS and resuspended in HBSS with 20 mM HEPES buffer (pH 7.4) with or without glucose (10 mM). Their viability estimated by Trypan blue staining was equal to 95%. They were then incubated (10^7 cells $\times$ ml^{-1}) at 37°C under aerobic conditions with the compounds to be investigated, and the cell suspension was used for lactate and ATP determination. Solvent controls (ethanol and dimethylsulfoxide) were included.

Thymocytes were isolated from the thymus of female Wistar rats (100–150 g), washed by HBSS and resuspended in the same medium. Their viability was about 90%.

Cell respiration was monitored by a Clark oxygen electrode at 37°C in HBSS. The RCR of intact cells was determined as described by Nakashima et al. [11], namely dividing the maximal respiratory rate in the presence of oligomycin + CCCP (state 3) by the respiratory rate in the presence of oligomycin only (state 4). Glycolysis was measured by lactate production; the latter was determined in the incubation medium with lactate dehydrogenase [13]. ATP was determined by the luciferine–luciferase method with a LKB Wallac 1251 luminometer.

CCCP and HEPES were from Sigma; rotenone and DNP were from Serva; oligomycin and lactate dehydrogenase were from Reanal, the ATP assay kit was from Calbiochem; all other reagents were of analytical grade.

The data shown are means ± S.E.M. of 3–8 independent determinations.

3. RESULTS AND DISCUSSION

EL-4 ascites thymoma, as with other rapidly growing tumors, has a high aerobic glycolysis and decreased Pasteur effect (about 40%) (Table I). However, glucose had much smaller effect on EL-4 cell respiration (Table I) than in EAC where respiration was inhibited about 2-fold (data not shown). The endogenous and glucose-supported respirations of EL-4 cells were almost totally (>95%) inhibited by rotenone, an inhibitor of NADH dehydrogenase (Table I) and by 1 mM cyanide, an in-

 Published by Elsevier Science Publishers B.V.

Table I

Effects of glucose and inhibitors of oxidative phosphorylation on respiration, glycolysis and ATP levels in EL-4 cells

Conditions	Respiration (nM O_2/min 10^6 cells^{-1})	Glycolysis (nM lactate/min·10^6 cells^{-1})	ATP (% of initial)	
			0.5 h	1 h
Control	0.67 ± 0.06	0.11 ± 0.01	70 ± 9	75 ± 2
Rotenone	0.04 ± 0.02	0 08 ± 0 03	4.7 ± 0.3	4.0 ± 0.7
DNP	1 23 ± 0.03	–	3 1 ± 0.2	–
CCCP	1.13 ± 0.04	–	2.0 ± 0 1	–
Oligomycin	0.15 ± 0.01	–	3.7 ± 0.1	–
Glucose	0.54 ± 0.04	1.95 ± 0,04	–	92 ± 6
Glucose + rotenone	0.04 ± 0.02	3 20 ± 0 20	–	84 ± 3
Glucose + DNP	0.86 ± 0.02	7.00 ± 0.40	–	81 ± 11

The cells were incubated in HBSS at 37°C; respiration, glycolysis and ATP levels were determined as described in section 2. Concentrations used: rotenone 2 μM; DNP 250 μM; CCCP 4 μM; oligomycin 1 μg/ml; glucose 10 mM. The initial ATP level was 15 ± 2 nM/10^6 cells.

hibitor of cytochrome oxidase (data not shown). An inhibitor of mitochondrial H^+-ATPase, oligomycin, also significantly (>70%) suppressed endogenous respiration, indicating its tight coupling to phosphorylation (Table I). Uncouplers (DNP and CCCP) stimulated endogenous respiration about 1.7–1.8-fold. The endogenous respiration could maintain cell ATP levels for 1 h and all inhibitors of oxidative phosphorylation caused rapid ATP depletion, which was totally prevented by glucose (Table I). The endogenous glycolysis of these cells was very low, so only exogenous glucose could maintain high ATP levels in these cells.

Thus, the above data show that endogenous respiration, as well as glycolysis, are effective in the ATP production in the studied cells. However, aerobic glycolysis in EL-4 cells had no inhibitory effect on cell respiration in contrast to EAC (see above) and many other tumor cells [1]. Such an effect may be due to the following possible reasons: increased ATPase activity in glucose-respiring cells and (or) decreased efficiency of phosphorylation.

An inhibitor of Na^+,K^+-ATPase, 1 mM ouabain, and oligomycin were found to be without effect on aerobic glycolysis in EL-4 cells (data not shown), although in some tumor cells they suppressed this process [3,4]. Thi may indicate that at least two ATPases with high activ ity (Na^+,K^+-ATPase and H^+-ATPase) do not play an significant role in the high aerobic glycolysis of thes cells. We also tested another possibility, and found th the RCR of EL-4 cells was significantly (about 2-fol decreased in the presence of glucose. As shown in Tab II, the RCRs of EL-4 cells were 6.2 with endogenou substrates and 8.0 with glutamine, but glucose d creased this value to 3.2, mainly by stimulating the sta 4 respiration. This effect was absent in the normal cou terpart of thymoma cells, thymocytes: their RCR w about 7.0, independent of glucose (Table II). The sam RCR of intact thymocytes was found previously [14]. similar lack of effect of glucose on the state 4 respirati was also found in EAC: their RCR was 7.2 (data n shown).

Thus, here we describe a new mechanism of increas aerobic glycolysis in tumor cells: glucose-induced lowe ing of the efficiency of oxidative phosphorylation. Th effect was found in EL-4 thymoma but not in norm cells (thymocytes) or in EAC cells showing significa Crabtree effect. The above finding may also explain t small Crabtree effect in EL-4 cells: their respiration

Table II

Effect of glucose on the RCR of EL-4 tumor cells and thymocytes (Thym.)

Substrate	Respiration (% of control)					
	Oligomycin		Oligomycin + CCCP		RCR	
	EL-4	Thym.	EL-4	Thym	EL-4	Thym.
Endogenous	23 ± 1	26 ± 5	142 ± 9	189 ± 19	6.2 ± 0.4	7.3 ± 0.7
Glucose	39 ± 3*	29 ± 4	124 ± 11	202 ± 20	3.2 ± 0.5*	7.0 ± 0.7
Glutamine	24 ± 2	–	193 ± 20	–	8.0 ± 0.8	–

All parameters were determined as described in section 2. Concentrations: glucose 10 mM, glutamine 4 mM, oligomycin 1 μg/ml, CCCP 2 μ The respirations in controls for EL-4 thymoma and thymocytes were 0.67 ± 0.06 and 0.11 ± 0.008 nM/min 10^6 cells, respectiv *$P < 0.01$ comparing with endogenous respiration.

partially uncoupled and cannot be inhibited by glycolysis, in contrast to EAC cells. As found previously, uncouplers were able to prevent the Crabtree effect in tumor cells [1]. Resting thymocytes had no significant aerobic glycolysis, so their respiration was not inhibited by glucose (data not shown).

The significance of this uncoupling effect of glucose in other tumor cell lines has to be evaluated, but recent data on the energetics of P388 leukemia cells may also indicate a low efficiency of their oxidative phosphorylation in glucose-containing media: the respiration in oligomycin-treated cells was 44% of controls [15] (cf. Table II).

Why does glucose increase the state 4 respiration? One possible explanation may be the following. It has been shown that endogenous respiration of tumor cells is due to fatty acids oxidation. Glucose significantly decreased this process, stimulating fatty acid synthesis [1,8]. So, addition of glucose may increase endogenous free fatty acid levels. As was shown previously, a low concentration (10 μM) of palmitate significantly decreased the RCR of isolated skeletal muscle mitochondria due to stimulation of the state 4 respiration [16,17]. A much higher content of endogenous free fatty acids was found in tumor mitochondria than in liver [18], and bovine serum albumin, which binds fatty acids, inhibited endogenous ATPase activities of human tumor mitochondria [19].

Acknowledgements I am grateful to Dr. S.Ya Proskuryakov for providing EL-4 and EAC cell cultures, and Drs. E.N. Mokhova, A.F. Mosin, and V.D. Sled for helpful discussions.

REFERENCES

[1] Aisenberg, A.S. (1961) The Glycolysis and Respiration of Tumors, Academic Press, New York, London.
[2] Pedersen, P.L. (1978) Progr. Exp. Tumor Res. 22, 190–274.
[3] Racker, E., Johnson, J.H. and Blackwell, M.T. (1983) J Biol. Chem. 258, 3702–3705.
[4] Kazmin, S.D. and Danko, I.M. (1989) Neoplasma 36, 139–147.
[5] Arora, K K and Pedersen, P.L. (1988) J. Biol. Chem. 263, 17422–17428.
[6] Bosca, L., Mojena, M., Ghysdael, J., Rousseau, G.G and Hue, L. (1986) Biochem J. 236, 595–599.
[7] Sauer, L.A. (1968) J. Biol. Chem. 243, 2429–2436.
[8] Sauer, L A. (1977) J Cell Physiol. 93, 313–316
[9] Medina, M.A., Sanchez-Jimenez, F., Marquez, F.J , Perez-Rodriguez, J., Quesada, A.R. and Nunez de Castro, I. (1988) Biochem Int. 16, 339–347
[10] Olavarria, J S., Galino, C., Montero, M., Baquero, Y., Vitorica, J. and Satrustegi, J. (1988) Biochim. Biophys. Acta 935, 322–332.
[11] Nakashima, R.A., Paggi, M.G and Pedersen, P L. (1984) Cancer Res. 44, 5702–5706.
[12] Gabai, V.L. and Mosin, A.F. (1991) Biokhimia 56, 1652–1660.
[13] Gutman, J. and Wahlefeld, A.W. (1974) Methods Enzymol. Anal. 3, 1464–1468.
[14] Konoshenko, G.I. and Mokhova, E N. (1983) Biokhimia 48, 652–657.
[15] Hyslop, P.A., Hinshaw, D.B., Halsey Jr., W.A., Schraufstatter, I.U., Sauerheber, R D., Spragg, R.G., Jackson, J.H. and Cochrane, C G. (1988) J. Biol. Chem. 263, 1665–1675.
[16] Skulachev, V.P. (1988) Membrane Bioenergetics, Springer-Verlag, Berlin.
[17] Andreev, A.Yu., Volkov, N.I., Mokhova, E.N. and Skulachev, V.P (1987) Biol. Membr. 4, 474–478.
[18] Barbour, R.L. and Chan, S.H.P. (1978) J. Biol. Chem. 253, 367–376.
[19] Knowles, A.F. and Kaplan, N.O. (1980) Biochim. Biophys. Acta 590, 170–181.