Biochimica et Biophysica Acta, 730 (1983) 271–275
Elsevier Biomedical Press

BBA 71648

THE EFFICIENCY OF ($Na^+ + K^+$)-ATPase IN TUMORIGENIC CELLS

ROBERT S. BALABAN[a,*] and JOHN P. BADER [b]

[a] *Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute and* [b] *Laboratory of Molecular Oncology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205 (U.S.A.)*

(Received September 24th, 1982)

Key words: ($Na^+ + K^+$)-ATPase; ATP turnover; K^+ pump; Oxygen consumption; (Tumor cell)

The efficiency of ($Na^+ + K^+$)-ATPase (i.e. the amount of K^+ pumped per ATP hydrolyzed) in intact tumorigenic cells was estimated in this study. This was accomplished by simultaneously measuring the rate of ouabain-sensitive K^+ uptake and oxygen consumption in tumorigenic cell suspensions during the reintroduction of K^+ to K^+-depleted cells. The ATP turnover was then estimated by assuming 5.6–6 ATP/O_2 as the stoichiometry of NADH-linked respiration in these cells. In the three cell lines tested (hamster and chick embryo cells transformed with Rous sarcoma virus and Ehrlich ascites cells), the K^+/ATP ratio was approximately 2, the same value as that found in normal tissues. Furthermore, only 20% of the total ATP production of these cells was used by ($Na^+ + K^+$)-ATPase.

Introduction

($Na^+ + K^+$)-ATPase plays a key role in the ionic homeostatis of most mammalian cells. In most cells this pump has been demonstrated to translocate 3 Na^+ out of the cell and 2 K^+ into the cell for every ATP hydrolyzed. However, some reports in tumorigenic cells suggest that ($Na^+ + K^+$)-ATPase is less efficient (i.e., translocates fewer ion per ATP hydrolyzed) [1–3], and therefore may consume a large fraction of the ATP produced in these cells.

Harris et al. [4] had previously reported a method of determining the efficiency of ($Na^+ + K^+$)-ATPase in intact cell suspensions based on the simultaneous measurement of oxygen consumption and ouabain-sensitive K^+ uptake into K^+-depleted cells. Using this approach we attempted to measure the efficiency of ($Na^+ + K^+$)-ATPase in intact tumor cells, and establish what percentage of the total ATP production of these cells was used by this homeostatic process.

Materials and Methods

The experimental method involved the simultaneous determinations of ouabain-sensitive K^+ uptake and oxygen consumption in three tumorigenic cell lines. Cells from a hamster tumor (HTC-BH) induced by the Bryan strain of Rous sarcoma virus (RSV-BH) were propagated in spinner cultures using 10% fetal bovine serum, 10% tryptose phosphate broth and Eagle's minimum essential medium containing sodium pyruvate and glucose. Chick embryo cells transformed by RSV-BH were grown on plastic dishes using a similar medium containing 5% fetal bovine serum. On the day before use, these cells were dispersed with trypsin and placed in spinner culture to allow cells to regenerate surface components. Ehrlich ascites cells

* To whom correspondence and reprint requests should be addressed: Laboratory of Kidney and Electrolyte Metabolism, Building 10, Room 6N307, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20205, U.S.A.

were grown intraperitoneally in mice. The rate of ouabain-sensitive K^+ uptake was determined from the rate of K^+ uptake measured after an injection of KCl into K^+-depleted cell suspensions [4]. All cell suspensions were K^+ depleted by washing the cells five times in K^+-free medium with 10 to 20 min incubation at 37°C between each wash.

The experimental chamber consisted of a water-jacketed glass cylinder, with pH and oxygen electrodes and an injection port mounted in the side of the cylinder (Fig. 1). The K^+ influx was measured with an extracellular K^+-selective electrode (ORION) inserted in the top of the chamber, making a gas tight seal. The K^+ electrode was calibrated by several injections of known KCl solutions into the incubation medium before and after each experimental series. The half-time of the electrode was approx. 1 s in this chamber for K^+ concentrations from 0.1 to 5 mM. Oxygen consumption was determined polarigraphically with an Instech model 125/05 oxygen electrode. Lactate production by the cells was measured in a glucose containing medium by measuring the total lactate content of aliquots of the suspensions as a function of time. Lactate was measured according to the method of Lowry and Passonneau [5]. The chamber volume was variable from 2.5 to 5.0 ml.

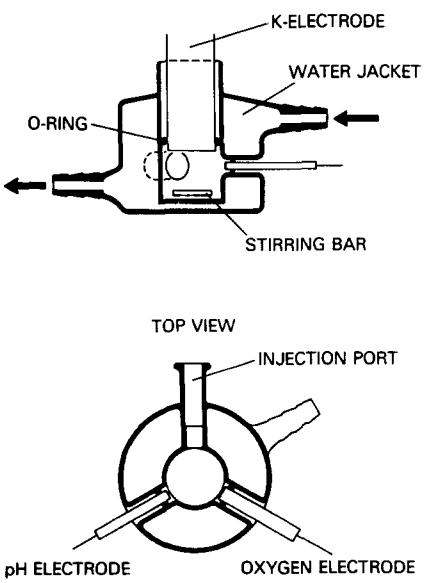

Fig. 1. Schematic drawing of the experimental chamber used in these studies.

The cell viability was judged by the linearity of oxygen consumption, and Trypan blue exclusion tests after an experimental run. Cell viability was routinely greater than 95% as judged from trypan blue exclusion, and oxygen consumption was linear over the 4- to 5-min experimental period. Cellular protein concentrations determined using the method of Bradford [6]. Cell suspensions between 1 and 6 mg protein/ml were routinely used. At these concentrations the cell volume made up between 2 and 12% of the total suspension volume. The control medium consisted of 115 mM NaCl, 25 mM $NaHCO_3$, 4 mM KCl, 10 mM glucose, 10 mM NaHepes, 0.5 mM $MgSO_4$, 1 mM CaCl, 5 mM NaH_2PO_4-Na_2HPO_4. In K^+-free media, KCl was replaced by NaCl. In glucose-free media, glucose was replaced by sodium lactate.

Results

Tumor cells possess very high rates of aerobic glycolysis [7]. In order to establish the relative contributions of aerobic glycolysis and mitochondrial respiration to ATP production in these cells, we measured the rate of lactic acid production and QO_2 in the presence of glucose or lactate. Table I presents the calculated ATP production rates of HTC-BH cells resulting from aerobic glycolysis and mitochondrial respiration in the presence of either 10 mM glucose or 10 mM lactate as substrates. The glycolytic ATP production in the presence of glucose was estimated by assuming 1 ATP per lactate produced and 0.4

TABLE I

ATP PRODUCTION IN HTC-BH CELLS

Condition	ATP production (nmol/min per mg protein)		
	Mitochondrial	Glycolytic	Total
Glucose	105 ± 6.2	50 ± 1.1	~155
Lactate	148 ± 8.2	~0	~148
Lactate + CCCP	299 ± 20.7 [a]	~0	

[a] Since CCCP stops mitochondrial ATP production, this value represents the ATP production rate which would correspond to this QO_2.

ATP/O_2 consumed. Lactate production was undetectable in the absence of exogenous glucose. The mitochondrial ATP production was calculated assuming 5.6 ATP/O_2 consumed. With glucose as the sole substrate, 33% of the ATP production originated from aerobic glycolysis, while the remaining 66% was from mitochondrial respiration. Similar results have been reported for Ehrlich ascites tumor cells [8]. As shown in Table I, mitochondrial ATP production increased with lactate as the sole substrate, to provide the same ATP production rate as the combined output of glycolysis and respiration in the presence of glucose. Furthermore, the rate of QO_2 in the presence of lactate was only 50% of the maximal rate determined in the presence of a mitochondrial uncoupler, carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazine (CCCP). Therefore, aerobic respiration alone was capable of producing sufficient ATP for normal metabolic processes without approaching its maximal rate. We chose to eliminate glycolysis as a source of ATP so that the ATP production rate could be accurately determined from the single measurement of oxygen consumption. This was accomplished by incubating the cells with lactate as the only exogenous substrate.

The simultaneous rates of K^+ uptake and O_2 consumption (QO_2) in HTC-BH cells are shown in Fig. 2a. The extracellular K^+ concentration was 0.45 mM prior to the injection of KCl, due to the leakage of K^+ from the cells into the originally K^+-free medium. KCL was injected, increasing the concentration to 2.1 mM, and the rate of K^+ uptake was determined from the linear portion of the first 20 s of the K^+-electrode trace as described earlier [4]. The average rate of uptake was 45.3 ($\pm$ 1.5) nmol K^+/min per mg protein ($N = 9$). Concomitantly QO_2 increased about 15%. In all cases, the QO_2 and the K^+ uptake rate used for the calculation of the K^+/O_2 ratio were from the same time interval. Upon reaching anoxia, caused by the depletion of O_2 in the sealed chamber, K^+ was released from the cells. In other experiments, K^+ uptake after injection of KCl was inhibited by anoxia or by antimycin A(10^{-5} M).

Addition of ouabain ($5 \cdot 10^{-4}$ M) prior to the addition of KCl caused an immediate increase of K^+ in the medium, and prevented K^+ uptake upon the injection of KCl (Fig. 2B). Addition of

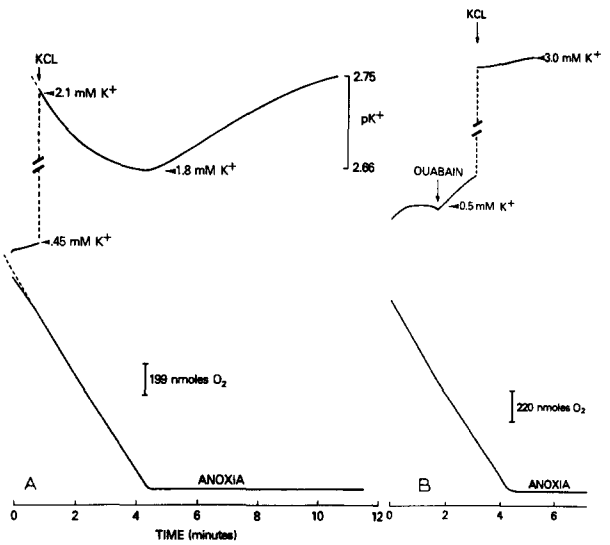

Fig. 2. K^+ uptake and QO_2 in HTC-BH cells. The upper trace is the K^+ electrode output, and the lower trace is the oxygen electrode output. (A) From a starting [K^+] of 0.45 mM the [K^+] was increased by an injection of 4 μl of 1.0 M KCl at the time indicated to bring the external K^+ concentration above 2 mM. Net K^+ uptake amounted to 55.2 nmol K^+/min per mg protein over the first 12 s. QO_2 increased from 25 to 29.7 nmol O_2/min per mg. The calculated K^+/O_2 ratio was 11.7, corresponding to a K^+/ATP ratio of 2.1 assuming 5.6 ATP/O_2. Anoxia resulted in the release of K^+ from the cells. The cellular protein concentration in this experiment was 2.0 mg protein/ml. (B) The addition of $5 \cdot 10^{-4}$ M ouabain caused the release of K^+ in the control condition. The subsequent injection of KCl caused no net K^+ uptake. In other experiments, ouabain added after an injection of KCl stopped net K^+ uptake and caused the net release of K^+ from the cells. The cellular protein concentration in this experiment was 1.9 mg protein/ml.

10^{-5} M bumetanide, a potent inhibitor of KCl cotransport, had no effect on the rate of K^+ influx in HTC-BH or Ehrlich ascites tumor cells using this experimental protocol. Because the uptake of K^+ was ouabain sensitive, and dependent on ATP synthesis, it was reasonable to assume that K^+ uptake was the result of ($Na^+ + K^+$)-ATPase activitiy.

It was important to establish that aerobic respiration was the major source of ATP production in this analysis. Thus, an inhibitor of glycolysis, iodoacetic acid, was used to estimate the contribution of glycolysis in the absence of external glucose. Pretreatment with iodoacetic acid ($5 \cdot 10^{-4}$ M) had little or no effect on QO_2 or K^+

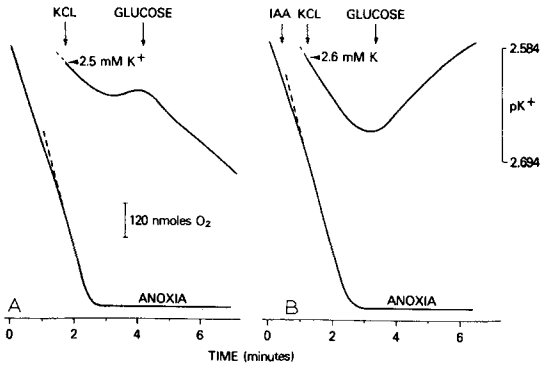

Fig. 3. K^+ uptake and QO_2 in Ehrlich ascites tumor cells. The upper trace is the K^+ electrode output, and the lower trace is the oxygen electrode output. (A) The starting $[K^+]$, omitted from this figure for clarity, was 0.43 mM. The injection of KCl caused a net K^+ uptake of 59.6 nmol K^+/min per mg protein in the first 15 s. This was associated with an increase in QO_2 from 26.4 to 31.8 nmol O_2/min per mg. The calculated K^+/O_2 ratio was 11, corresponding to a K^+/ATP ratio of 1.95 assuming 5.6 ATP/O_2. Anoxia stopped net K^+ uptake but was reinitiated by an injection of 10 mM glucose. The cellular protein concentration was 5.4 mg/ml. (B) The starting $[K^+]$ in this experiment was 0.5 mM. The injection of iodoacetic acid (IAA) had no effect on the steady-state $[K^+]$ (not shown) or QO_2. Under these conditions a KCl injection resulted in a K^+ uptake of 57.2 nmol K^+/min per mg in the first 15 s and increased QO_2 from 26.3 to 31.0 nmol O_2/min per mg protein. The calculated K^+/O_2 ratio was 12.2, corresponding to 2.2 K^+/ATP assuming 5.6 ATP/O_2. During anoxia, glucose (10 mM) did not reinitiate K^+ uptake in the presence of iodoacetic acid. The cellular protein concentration was 5.5 mg/ml.

uptake in the presence of oxygen. In order to make sure that iodoacetic acid inhibited glycolysis, glucose was added to Ehrlich ascites tumor cells during anoxia. In the absence of iodoacetic acid, glucose reinitiated K^+ uptake (Fig. 3A) presumably by providing ATP through an aerobic glycolysis. Iodoacetic acid prevented the glucose-stimulated K^+ uptake during anoxia (Fig. 3B), demonstrating that iodoacetic acid effectively blocked the production of ATP by glycolysis. Ouabain ($5 \cdot 10^{-4}$ M) also blocked the glucose-stimulated K^+ uptake, confirming that $(Na^+ + K^+)$-ATPase was required for this K^+ uptake. These data demonstrate that glycolysis makes an insignificant contribution of the production of ATP for $(Na^+ + K^+)$-ATPase when cells are incubated with lactate in the absence of glucose.

The instantaneous rate of K^+ uptake and QO_2 in response to exogenous KCl can be used to calculate a K^+/O_2 ratio (Table II), and from this a K^+/ATP ratio can be calculated assuming a value for the ATP/O_2 ratio during NADH-linked respiration. These is a small uncertainty about the absolute value of the ATP/O_2 ratio of respiration, since the classical integer value of 6 differs slightly from the experimentally observed value of 5.6 found both in intact Ehrlich ascites cells [9] and in isolated mitochondria [4,10]. In either case, the K^+/ATP ratio was approximately 2 for each of the three cell types examined.

Discussion

These results are consistent with a K^+/ATP stoichiometry of 2 for $(Na^+ + K^+)$-ATPase mediated K^+ uptake in the three tumorigenic cell lines we studied after K^+ depletion. This K^+/ATP

TABLE II

THE K^+/ATP RATIO FOR THREE TUMORIGENIC CELL LINES

CE-BH = chick embryo cells transformed by Rous sarcoma virus; HTC-BH = cells from hamster tumor induced by Rous sarcoma virus; EA = Ehrlich ascites tumor cells. The K^+ uptake was measured as discussed in the text. The ΔQO_2 was calculated by substracting the control QO_2 from the QO_2 after the injection of KCl. The K^+/ATP ratios were calculated using ATP/O_2 ratios of both 6 and 5.6.

Cell type	K^+-uptake rate (nmol/min per mg protein	ΔQO_2	K^+/O_2	K^+/ATP		N
				ATP/O_2 = 6	ATP/O_2 = 5.6	
CE-RSV BH	36.9 ± 4.1	3.2 ± 0.4	11.4 ± 0.2	1.9 ± 0.1	2.0 ± 0.1	5
HTC-BH	43.2 ± 2.5	3.7 ± 0.3	12.2 ± 0.9	2.0 ± 0.2	2.2 ± 0.2	11
EA	53.0 ± 4.9	4.95 ± 0.6	11.2 ± 0.6	1.9 ± 0.1	2.0 ± 0.1	12

stoichiometry of 2 is essentially the same as those found in normal tissues such as erythocytes [11,12] and especially renal cortical tubules studied under identical conditions [4]. Thus, $(Na^+ + K^+)$-ATPase is apparently no less efficient in the tumorigenic cell lines we have studied than in normal tissue under these conditions. The rate of ATP production in HTC-BH cells was approx. 150 nmol/min per mg protein, while the unindirectional K^+ influx was approx. 43.0 nmol of K^+/min per mg protein, which corresponds to only 18.5 nmol ATP/min per mg protein assuming 2 ATP/K^+. Thus, this turnover of $(Na^+ + K^+)$-ATPase apparently consumes only 15 to 20% of the ATP produced by the cells. This contention is supported by the observation that ouabain ($5 \cdot 10^{-4}$ M) inhibited QO_2 by only 20% ± 1.2 ($N = 12$) when added to HTC-BH cells in the presence of 2 mM KCl and lactate as the sole substrate. These latter results are similar to those found for Ehrlich ascites tumor cells [8].

In summary, the efficiency of $(Na^+ + K^+)$-ATPase in the three cell lines investigated is identical to that found in normal cells. Furthermore, in the HTC-BH cells, the turnover of $(Na^+ + K^+)$-ATPase apparently consumes only 20% of the total ATP production.

Acknowledgement

We than Nancy R. Brown and Joan Koudratick for their assistance in growing and processing the cells used in these studies.

References

1 Svolinna, E.M., Land, D.R. and Racker, E. (1974) J. Natl. Cancer Inst. 53, 1515–1519
2 Racker, E. (1976) J. Cell. Physiol. 89, 697–700
3 Svolinna, E.M., Buchsbaum, R.N. and Racher, E. (1975) Cancer Res. 35, 1865–1872
4 Harris, S.I., Balaban, R.S. and Mandel, L.J. (1980) Science 208, 1148–1150
5 Lowry, O.H. and Passonneau, J.V. (1972) A Flexible System of Enzymatic Analysis, Academic Press, New York
6 Bradford, M. (1976) Anal. Biochem. 72, 248–254
7 Warburg, O. (1956) Science 123, 309–314
8 Maizels, M., Remington, M. and Truscoe, R. (1958) J. Physiol. (London) 140, 80–93
9 Hess, B. and Chance, B. (1959) J. Biol. Chem. 234, 3031–3035
10 Hinkle, P.C. and Yu, M.L. (1979) J. Biol. Chem. 254, 2450–2455
11 Sen, A.K. and Post, R.L. (1964) J. Biol. Chem. 239, 345–352
12 Garrahan, P.J. and Glynn, I.M. (1967) J. Physiol. (London) 192, 217–235