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EFFECT OF K^+ DEFICIENCY ON OXIDATIVE METABOLISM IN EHRLICH ASCITES TUMOR CELLS

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SUMMARY

The effect of cell K^+ content, and cell-membrane K^+ transport on mitochondrial ATP generation was studied in Ehrlich ascites tumor cells.

1. Depletion of cell K^+ by repeated washings in cold K^+-free medium results in an initial decline in ATP with concomitant increase in ADP and AMP content on incubation at 37 °C. After prolonged incubation, ATP content falls to negligible levels and AMP degradation is evident as manifested by a fall in total adenine nucleotides. The fall in ATP is a consequence of reduced electron transport through the mitochondrial respiratory chain.

2. ATP reduction in the K^+-depleted cell can be prevented if the cells are incubated in a K^+-containing medium. Addition of K^+ after preincubation of K^+-depleted cells does not lead to restoration of adenine nucleotide levels. By contrast, addition of glutamine to K^+-depleted cells, with or without preincubation, restores the ATP content. This effect of exogenous substrate, and the unimpaired conversion of [^{14}C]palmitate to $^{14}CO_2$ suggest that K^+-depletion does not directly interfere with oxidative phosphorylation and that availability of endogenous substrates for mitochondrial oxidation is limiting in the K^+-deficient state.

INTRODUCTION

Cell membrane K^+ transport has been implicated in the normal operation of both glycolysis and respiration in mammalian tissues. This energy-dependent, K^+ transport system serves as a "pacemaker" for mitochondrial respiration[1] and directly influences the rate of glycolysis in erythrocytes[2,3] and in Ehrlich ascites tumor cells[4,5] probably at the phosphoglycerate kinase level[6,7]. The intracellular K^+ concentration has been assigned an important role in the regulation of the rate of glycolysis in Ehrlich ascites tumor cells[8,9], presumably through its effect on pyruvate kinase[10].

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A regulatory role for K^+ on mitochondrial metabolism in intact cells has been difficult to assess. The observations of a high K^+ concentration in mitochondria[11], an effect of K^+ on the permeability of isolated mitochondria to substrate[12-15], an effect on respiratory control[16] and the P.O ratio[17], and on adenine nucleotide translocase[18] has led to extrapolations bearing on the effects of K^+ deficiency on mitochondrial oxidative phosphorylation in the intact cell. It has been argued that the intracellular K^+ content is a more important determinant of oxidative metabolism than is cellular transport of K^+ (ref. 19). In the experiments presented here, the respiratory inhibition observed in K^+-deficient cells could be overcome by addition of exogenous substrate suggesting that K^+ deficiency exerts its effect by reducing the availability of substrate for mitochondrial oxidation. In the absence of a functioning Embden–Meyerhof pathway, K^+ deficiency leads to a reduction in cell ATP and ultimately to an irreversible decline in total cell adenine nucleotides.

METHODS

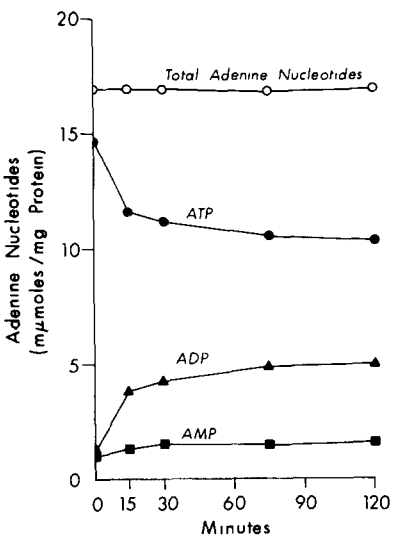
Ehrlich ascites tumor cells of the hyperdiploid strain were harvested from the peritoneal cavity of white mice, 6 to 8 days after inoculation. K^+-depletion was accomplished by washing the cells repeatedly in ice-cold K^+-free medium over a period of 3 h as previously described[20]. Non-depleted cells were similarly prepared except for the presence of 5 mM K^+ in all media.

Incubations were carried out in a Dubnoff metabolic shaker in an air atmosphere at 37 °C. The final cell protein concentration in the medium was approximately 4 mg/ml. At appropriate intervals aliquots of the cell suspension were removed and added to 6 % perchloric acid. The supernatant solution was neutralized to approximately pH 6.8 with 5 % KOH containing 0.15 M glycylglycine[21]. After removal of the potassium perchlorate precipitate by centrifugation, aliquots of the supernatant solution were assayed for adenine nucleotides[22,23]. The respiratory rates were determined with a Clark oxygen electrode at indicated intervals in a closed system at 37 °C. For K^+ determination, the cells were rapidly washed twice at room temperature with 0.154 M choline chloride, the washed cell pellet was digested over-night with 0.1 M HNO_3 and K^+ content was determined by flame photometry on the supernatant solution. Cell protein was determined by a biuret method with bovine serum albumin as the standard The [*carboxy*-^{14}C]palmitate (New England Nuclear Corp. Boston, Mass.)–albumin complex was prepared by the method of Lorch and Gey[24] using fatty acid-free albumin (Pentex, Miles Labs. Kankakee, Ill.); the molar ratio of palmitate to albumin was 4. The conversion of ^{14}C-labeled palmitate to $^{14}CO_2$ by Ehrlich ascites tumor cells was determined in stoppered Erlenmeyer flasks incubated at 37 °C. At the end of the incubation, perchloric acid was added and the flasks were shaken for an additional 30 min in order to insure entrapment of all $^{14}CO_2$ on KOH saturated filter paper in the center well. The filter paper and center well were washed with a large volume of water; carrier sodium bicarbonate was then added to the combined washings followed by barium chloride leading to the precipitation of the radioactive CO_2 as barium carbonate. The radioactivity of the plated barium carbonate was determined with a gas flow counter and the activity was corrected to infinite thickness.

RESULTS

Adenine nucleotides and respiration of K^+-depleted and non-depleted cells

The collection and washing of cells prior to incubation necessitates temperature adjustments which result in a consistent decline in the ATP content of cells during the first 15 to 30 min of incubation at 37 °C (Fig. 1) Following this initial decline, the ATP content as well as the total adenine nucleotide content of non-depleted cells remain relatively constant. In contrast, there is a steady decline of ATP in K^+-depleted cells (Fig. 2). Total adenine nucleotides (ATP+ADP+AMP) of K^+-depleted cells remain relatively constant for 30 min and subsequently exhibit a substantial decline (Fig. 2). An increase of AMP and ADP counter-balance the decrease in ATP during the first 30 min of incubation. Between 30 and 90 min, ADP content decreases along with ATP levels while AMP increases slightly indicating adenine nucleotide degradation. Incubation of K^+-depleted cells in a medium containing 5 mM K^+ prevents the decline in ATP; 5 mM Rb^+ or Cs^+ are somewhat less effective than K^+ in this regard.



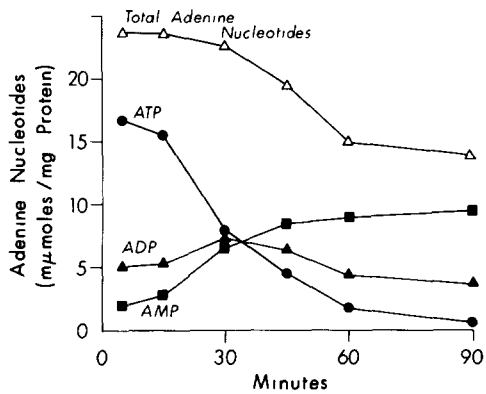
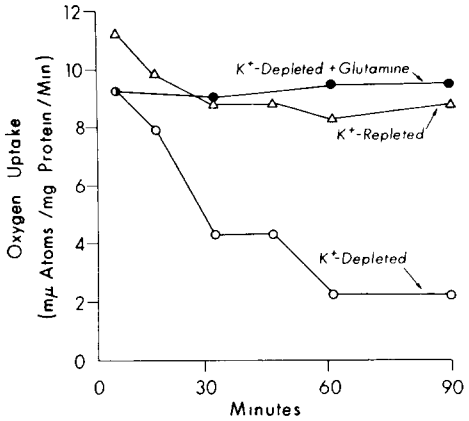


Fig 1 Adenine nucleotides in non-depleted Ehrlich ascites tumor cells. 5 mM K^+ was present in the collecting, washing, and incubation media.

Fig. 2 Adenine nucleotides in K^+-depleted Ehrlich ascites tumor cells.

In the absence of exogenous substrate, the oxygen uptake of depleted cells decreases significantly (Fig. 3); the early decline in respiration corresponds to the early fall in cell ATP. In K^+-repleted cells, the oxygen uptake decreases minimally during the first 30 min after which time a relatively constant rate of respiration is maintained. Since ATP production occurs only in mitochondria in the absence of exogenous substrate, the decline of oxygen uptake in the K^+-depleted cells corresponds to a decline in ATP generation.



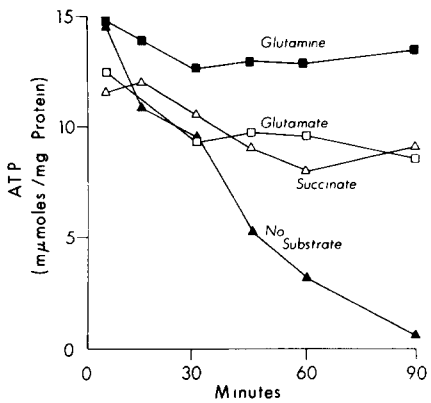


Fig 3 The respiratory rate of K^+-repleted and K^+-depleted cells in the presence and absence of 5 mM glutamine. K^+-repleted cells were first depleted of K^+ by the standard procedure and then incubated in the presence of 5 mM K^+.

Fig 4 Effect of exogenous substrates on maintaining the ATP content of K^+-depleted cells The concentration of substrates were glutamine 5 mM, sodium glutamate 10 mM, and sodium succinate 10 mM

Effect of exogenous substrate

Incubation of K^+-depleted cells in the presence of 5 mM glutamine, 10mM glutamate or 10 mM succinate prevents a decline in ATP content; glutamine is more effective than glutamate or succinate (Fig. 4). This difference may be due to the relative rates of penetration of each of these substrates across the cell[25] or mitochondrial membranes[26]. Addition of 5 mM glutamine to depleted cells preincubated for 30 min at 37 °C increases the ATP content from 4.8 nmoles of ATP per mg protein to 14 nmoles of ATP per mg protein, within 5–10 min The addition of glutamine to non-depleted cells abolishes the initial decrease in ATP that characteristically appears during the first 30 min. ATP content of non-depleted cells incubated in the presence of glutamine remains relatively constant at approximately 14 nmoles of ATP per mg protein. The respiratory rate of depleted cells remains unchanged when 5 mM glutamine is present in the incubation mixtures (Fig. 3). When K^+ is added to depleted cells after 30 min of incubation, no effect on the ATP content or respiration of the cells is observed and the cells do not concentrate K^+. Conceivably, the ATP content of the cell is too low to support the cell-membrane, energy-dependent, K^+ transport system[27,28] or alternatively, substrate availability is limiting.

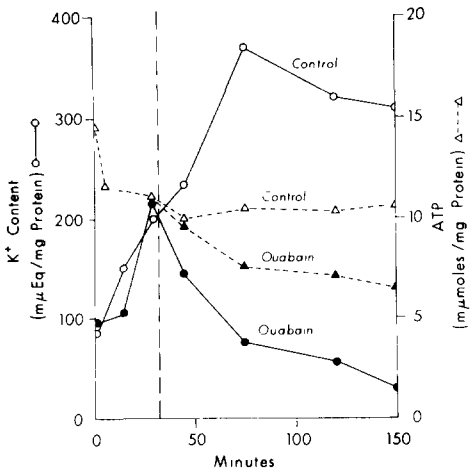
K^+ transport

After Ehrlich ascites tumor cells are removed and washed at 4 °C in a medium containing 5 mM K^+, the cell K^+ content is approximately 80 nequiv/mg cell protein; cells washed in the absence of K^+ contain approximately 5–10 nequiv/mg cell protein. Incubation of either of these cell preparations at 37 °C in a medium containing 5 mM K^+ results in the accumulation of intracellular K^+ (approximately 300 nequiv/mg protein) against a concentration gradient in agreement with earlier studies[29]. Since uptake of K^+ against a concentration gradient requires energy, the decrease in

ATP level during the first 30 min of incubation probably reflects the rapid utilization of ATP for K^+ transport into the cell. Other experiments indicate that changes in ATP content and in K^+ reaccumulation of non-depleted cells are independent of the duration of the washing procedure.

Influence of ouabain

Interference with the energy-dependent Na^+–K^+ pump by addition of 1 mM ouabain after intracellular K^+ is partially restored, results in a prompt, progressive decline in K^+ (Fig. 5). A significant decrease in cell ATP content is also observed, but is not as precipitous as that seen with K^+-depleted cells (Fig. 1). The largest fractional decrease in ATP content occurred during the first 45 min after addition of ouabain Inhibition of the K^+ transport system itself could therefore not account for the rapid decline in ATP to negligible levels in K^+-depleted cells.



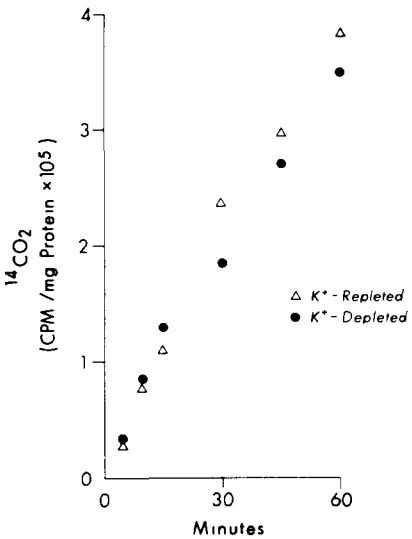


Fig 5. The effect of ouabain on the K^+ and ATP content Non-depleted cells were incubated for 30 min in order to increase the K^+ content; ouabain was added to one vessel in a final concentration of 1 mM and medium to the control vessel. The time of the additions is indicated by the vertical interrupted line.

Fig 6. The production of $^{14}CO_2$ in K^+-repleted and K^+-depleted cells in the presence of 0 046 mM [^{14}C]palmitate as the albumin complex.

Oxidation of fatty acids

Exogenous fatty acids added to incubating Ehrlich ascites tumor cells as the albumin complex are rapidly taken up and metabolized[30]. In order to determine whether K^+ deficiency influences fatty acid oxidation, Ehrlich ascites tumor cells were incubated with [^{14}C]palmitate and the formation of $^{14}CO_2$ was followed. The effect of K^+-depletion on [^{14}C]palmitate conversion to $^{14}CO_2$ is shown in Fig. 6. The rate of fatty acid oxidation in both repleted and depleted cells is linear with time and no difference is noted for the two types of preparations. These data suggest that neither

fatty acid transport into mitochondria, fatty acid oxidation, or tricarboxylic acid cycle activity is affected by the K^+-deficient state

DISCUSSION

A high concentration gradient for K^+ between intracellular and extracellular fluid exists for most mammalian tissues. The intracellular K^+ concentration is approximately 20-fold greater than that of the fluid bathing cells. A reduction in intracellular K^+ content is thought to influence energy-generating and biosynthetic pathways directly, or indirectly by restricting the cell membrane monovalent cation pump.

In the present experiments K^+ depletion induced by washing Ehrlich ascites tumor cells at low temperature in K^+-free medium[20] or by incubation in the presence of ouabain[31] leads to an initial marked reduction in ATP without substantial decline in total adenine nucleotides. The ATP decline is a consequence of restriction of mitochondrial energy generation as manifested by a decrease in cell respiration. Glycolysis plays no role in the ATP generation of Ehrlich ascites tumor cells unless glucose is added.

It is unlikely that the reduced ATP generation in K^+-depleted cells is primarily due to inoperability of the cell membrane Na^+–K^+ transport system. When pump activity is restricted by omitting K^+ from the incubation medium, ATP content and ATP generation can be maintained by addition of exogenous substrate, *e.g.* glutamine, succinate, or glutamate Further, inhibition of the (Na^+–K^+)ATPase with ouabain in non-depleted cells incubating in a K^+-containing medium does not affect energy generation[31].

Maintenance and restoration of the respiratory rates and ATP in K^+-depleted cells by exogenous substrate suggests that the intracellular K^+ concentration has little effect on the oxidation of substrate (glutamine, glutamate, succinate), the electron transport chain, or the conservation of energy. This conclusion is also supported by the almost identical conversion rates of [^{14}C]palmitate to $^{14}CO_2$ in control and K^+-deficient states. It is conceivable that the intramitochondrial K^+ concentration may be little affected despite reduction in overall cell K^+ content. The restoration of energy generation in K^+-depleted cells by exogenous substrate speaks against an effect of high intracellular Na^+ on mitochondrial ATP production.

It has been suggested that amino acids serve as the primary metabolic fuel for Ehrlich ascites tumor cells[26,32,33] rather than carbohydrate[34] or lipid[33]. If amino acids are an important metabolic fuel for Ehrlich ascites tumor cells oxidizing endogenous substrates, then intracellular K^+-deficiency might affect the respiratory rate by limiting substrate availability It has been established[35,36] that the efflux of glycine from ascites tumor cells is stimulated by an intracellular environment with high Na^+ concentration and low K^+ concentrations, a state that exists in the K^+-depleted Ehrlich ascites tumor cell. If this same mechanism were operative for other amino acids, K^+-depletion could lead to endogenous substrate depletion. The data presented in this report confirm previous observations suggesting that intracellular K^+-deficiency affects oxidative metabolism. The site affected by low K^+ in Ehrlich ascites tumor cells is proximal to the oxidative sequence of enzymes; the data point to a limitation of substrate availability.

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