The Effect of Different Incubation Temperatures on the Adenine Nucleotide Content of Ehrlich-Lettré Ascites Tumor Cells

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Summary

Ehrlich-Lettré ascites tumor cell suspensions incubated under air at 20° showed a decrease in ATP and total adenine nucleotide after the addition of 10 mM glucose. Duplicate portions of the same cell suspensions incubated at 37° did not show these changes after glucose addition. The decrease in the adenine nucleotide pool which occurs at 20° appears to be the result of a decreased rate of AMP rephosphorylation. As a consequence, AMP accumulates and is removed from the adenine nucleotide pool by adenylate deaminase. At 37°, the rate of AMP rephosphorylation is rapid, AMP does not accumulate, and there is no decrease in the total adenine nucleotide pool.

The critical temperature for this phenomenon occurs at about 30°. Below this temperature 10 mM glucose caused a decrease in both ATP and total adenine nucleotide. Above this temperature glucose had no effect on the adenine nucleotides. The extent of the adenine nucleotide degradation which occurs at the lower incubation temperatures is dependent on the glucose concentration added. Glucose concentrations below 0.5 mM did not cause an appreciable degradation of ATP or total adenine nucleotide. These temperature-dependent changes in the adenine nucleotides may become important considerations in in vitro studies since it is common practice to perform experiments with ascites tumor cell suspensions at temperatures which range from 20 to 38°.

Introduction

The total adenine nucleotide content of ascites tumor cells is kept constant by a balanced action of enzymes which degrade and synthesize AMP. During endogenous respiration, the relative proportions of the individual nucleotides are controlled by oxidative phosphorylation and the enzyme adenylate kinase. These reactions maintain the low concentrations of ATP and ADP and the high concentration of ATP. Removal by adenylate deaminase of AMP from the adenine nucleotide pool is inhibited apparently by a low substrate concentration, and perhaps by other allosteric controls (14) as well. Under certain conditions this delicate balance may be disrupted. For example, during massive ATP dephosphorylation and impaired glycolysis [such as that which follows the addition of 2-deoxyglucose (7), glucose plus iodoacetate (16), or glucose plus oxamate (9)], there is an elevation of and then a decrease in AMP and ADP. This is followed by a decrease in ATP and AXP. The disruption of the energy pool has a profound and prolonged effect on the ability of the ascites cell to form ATP (17). During the usual in vitro incubation (performed in the absence of these glycolytic inhibitors), a stable intracellular adenine nucleotide content is assumed and, indeed, may be crucial for the reaction under investigation. Therefore, the finding that the addition of glucose alone may lead to similar changes in the adenine nucleotides of ascites tumor cells (5, 8, 12) is important. While investigating the Crabtree effect in ELD ascites tumor cells, we observed a glucose-induced degradation of both the ATP and the total adenine nucleotides which was dependent on the temperature of incubation. At temperatures below about 30° there was a decrease in the ATP and the AXP. In similar incubations performed at 37° this degradation did not occur. This observation may have importance for in vitro studies, since it is common practice to incubate glucose-fortified ascites tumor cell suspensions at various temperatures ranging from 20° (3), 23° (6), 26° (1), and 30° (16) to 38° (4). In this report we describe some of the properties of the temperature-dependent adenine nucleotide degradation.

Materials and Methods

The ELD ascites tumor cells used in these experiments were originally obtained from Dr. T. S. Hauschka, Roswell Park Memorial Institute, Buffalo, N. Y., and have been maintained in this laboratory through some 80 transplants. Tumor transplantation and harvest (13) and oxygen consumption measurements (11) were performed as previously described. All incubations were done in a Tris-Ringer solution containing 20 mM Tris-HCl, 6 mM KCl, and 0.9% NaCl. This solution was adjusted to pH 7.4 at 25°. The small pH change which resulted from the use of this medium at 20 and 37° did not seem important, since the same results were obtained when using a Tris-Ringer solution adjusted to pH 7.4 at 20 and 37°. The adenine nucleotides were measured spectrophotometrically or fluorometrically in KOH-neutralized perchloric acid extracts.

1 This research has been partially supported by USPHS Grant AM-05954-07.
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Received October 24, 1969; accepted March 4, 1970.

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Glucose was measured with Glucostat reagents obtained from Worthington Biochemical Corporation, Freehold, N. J. Protein was determined by the biuret method (15), and all chemicals used were reagent grade.

RESULTS AND DISCUSSION

Chart 1 shows the changes in the adenine nucleotides caused by the addition of 10 mM glucose to an ELD ascites tumor cell suspension. In this experiment equal portions of the same cell suspension were incubated at 20 and 37°C. Glucose was added at 0 time. A transient increase in ADP and AMP and a transient decrease in ATP may be seen at 37°C if samples are removed at 15 and 30 sec after glucose addition (16). These changes, however, are completely reversible and were not seen 1 min after glucose addition. An irreversible decrease in ATP and the AXP occurred only in the 20°C incubations. The maximum decrease in ATP was reached about 3 min after glucose addition. The ATP was not replenished during a 10-min incubation (Chart 1) and only slightly after a 20-min one (not shown). The effect observed at 20°C was very reproducible. For example, in 4 experiments performed as shown in Chart 1, the ATP and AXP contents (±1 S.E.) in cells incubated at 20°C were 14.6 ± 0.9 and 18.6 ± 0.7 mmoles/mg protein, respectively, 2 min before glucose addition. Six min after glucose addition, the ATP and AXP contents of these cells had decreased to 9.9 ± 1.0 and 15.2 ± 0.5 mmoles/mg protein, respectively. In identical portions incubated at 37°C, the ATP and AXP contents were 15.2 ± 0.6 and 19.0 ± 1.2 mmoles/mg protein 2 min before glucose addition and 15.6 ± 0.9 and 19.2 ± 1.3 mmoles/mg protein, respectively, 6 min after glucose addition. In several other experiments performed at other temperatures between 20 and 30°C, decreases in ATP and AXP content were also observed (not shown). A comparison of the results of these experiments suggests that the amount of the ATP and AXP degradation increases as the incubation temperature decreases. However, there are differences between cell preparations, and more experiments are required to determine the reliability of this observation.

The amount of the ATP and AXP decrease which occurred in the incubations below 30°C was dependent on the amount of glucose added (Table 1). A glucose concentration high enough to maintain a continual glucose supply throughout the incubation was necessary. Low added glucose concentrations were completely utilized during the brief, rapid period of glucose uptake (12, 16) and apparently did not cause increased rates of ATP dephosphorylation and AMP formation.

It is remarkable that incubation temperatures which differ by 10–15°C may have different effects on the size and composition of the adenine nucleotide pool. These data suggest that this difference may be explained by the accumulation of AMP which occurs at the lower incubation temperatures. At 37°C, the AMP released following glucose addition is kept within the adenine nucleotide pool by rapid rephosphorylation to ADP and ATP. At lower incubation temperatures, one would expect slower rephosphorylation rates, higher steady-state AMP levels, and, therefore, increased AMP degradation.

Table 1

<table>
<thead>
<tr>
<th>Initial glucose concentration (mM)</th>
<th>Adenine nucleotides</th>
<th>Final glucose concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>ATP 15.5 ADP 2.4 AMP 1.4 AXP 19.3</td>
<td>None</td>
</tr>
<tr>
<td>0.1</td>
<td>15.1 ADP 2.3 AMP 1.4 AXP 18.8</td>
<td>0.03</td>
</tr>
<tr>
<td>0.17</td>
<td>13.6 ADP 2.5 AMP 1.1 AXP 17.2</td>
<td>0.03</td>
</tr>
<tr>
<td>0.5</td>
<td>8.1 ADP 2.7 AMP 2.9 AXP 13.7</td>
<td>0.24</td>
</tr>
<tr>
<td>1.7</td>
<td>4.7 ADP 2.7 AMP 3.1 AXP 10.5</td>
<td>1.33</td>
</tr>
<tr>
<td>5.0</td>
<td>3.7 ADP 3.6 AMP 3.6 AXP 10.9</td>
<td>4.45</td>
</tr>
</tbody>
</table>

An ELD cell suspension in Tris-Ringer solution (pH 7.4) containing 5.5 mg protein/ml was incubated at 25°C in an oxygen electrode chamber. Two min after a linear endogenous respiratory rate had been achieved, glucose was added. Five min later a 2-ml portion was removed for glucose and adenine nucleotide analysis. The adenine nucleotides are expressed as mmoles/mg protein. This particular cell suspension showed an exceptionally large ATP and AXP decrease.
Some evidence that this mechanism may explain the protective effect of high incubation temperatures is presented in Table 2. At 37° the rates of respiration and glucose consumption were increased 2- to 3-fold relative to those measured at 20°.

The calculated rates of ATP formation indicate that at 37° there is no overall decrease in ATP production as a result of the Crabtree effect. Glycolytic ATP production compensates for that ATP lost by the inhibition of oxidative phosphorylation. This finding agrees with the reports of Quastel and Bickis (10) and Yushok (17). At 20°, ATP formation from endogenous respiration is greatly decreased, and after glucose addition the rate of ATP formation is even further diminished. Glycolytic ATP formation is insufficient to replace that ATP lost due to the Crabtree effect. Below a certain temperature it may be that ATP resynthesis is unable to keep pace with ATP dephosphorylation and that AMP accumulates. This effect explains the ATP decrease that occurs the AMP is deaminated, and a decrease in the adenine nucleotide pool results. This effect explains the ATP decrease noted previously in ELD cell suspensions incubated at 25° (12). It may be that any physical change (lower incubation temperature) or chemical agent (glycolytic inhibitors) which diminishes the overall rate of ATP formation after glucose addition will lead to an elevated intracellular AMP concentration and an increase in AMP deamination. This effect would be especially pronounced in ascites tumor cells which possess an exceptionally active group of enzymes for the conversion of AMP to IMP and inosine (7).

Table 2
The effect of temperature on ATP formation by respiration and aerobic glycolysis in ELD ascites tumor cells

<table>
<thead>
<tr>
<th>Temperature and formation</th>
<th>Respiratory rate (μmoles O₂/mg/min)</th>
<th>Glucose uptake (μmoles/mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before glucose</td>
<td>After glucose</td>
</tr>
<tr>
<td>20°</td>
<td>4.2 ± 0.4</td>
<td>2.4 ± 0.5</td>
</tr>
<tr>
<td>ATP formation</td>
<td>25.2</td>
<td>14.4</td>
</tr>
<tr>
<td>37°</td>
<td>9.0 ± 0.3</td>
<td>5.9 ± 0.4</td>
</tr>
<tr>
<td>ATP formation</td>
<td>54.0</td>
<td>35.4</td>
</tr>
</tbody>
</table>

a These calculations are based on the assumptions that respiration is tightly coupled (a P/O ratio of 3) and that each glucose consumed leads to the rephosphorylation of 2 ATP's. ATP formation is in μmoles/mg/min. The values in parentheses are the ATP formations during aerobic glycolysis. In these calculations, glucose consumption due to the hexose monophosphate shunt was assumed to be 0 at both temperatures. Appreciable glucose uptake through this pathway would further diminish calculated ATP formation by aerobic glycolysis.

On the basis of these experiments, it appears that the results of in vitro metabolic studies performed with ELD ascites tumor cells at different temperatures may not be directly comparable. This is especially true for Crabtree effect studies in which high glucose concentrations are added to preincubated cell suspensions. At the present, we do not know if a temperature-dependent adenine nucleotide degradation occurs in other Ehrlich ascites tumor cell strains. There may be strain differences, since an adenine nucleotide degradation occurred at 37° in dilute suspensions of Ehrlich-Landshutz hyperdiploid ascites tumor cells (8).

Despite the different temperatures and the adenine nucleotide degradation which occurred at 20°, a Crabtree effect was evident (Table 2) at both incubation temperatures. An interesting possibility raised by these experiments is that in ELD ascites tumor cells the Crabtree effect at 20—25° may have a mechanism different from that at 37°. This is currently under study.

REFERENCES


Temperature-dependent AXP Changes


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