Short-term effect of glutamine on the incorporation of glucose-carbon into nucleic acids in Ehrlich ascites tumour cells

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Summary — A slight but significant short-term incorporation (15 and 60 min) of glucose-carbon into DNA and RNA was detected in Ehrlich ascites tumour cells incubated in the presence of labelled glucose (5 mM). Glutamine (0.5 mM) increased the incorporation of glucose but did not modify the glycolytic flux.

Glutamine / glucose / nucleic acids / Ehrlich tumour cells

Introduction

Glucose is the main energy substrate for Ehrlich ascites tumour cells [1]; in short-term batch incubations, only a small amount is completely oxidized to CO₂. However, in perfused fed cells after 50 min of continuous perfusion, a steady state is reached, in which glucose is stoichiometrically converted into 2 moles of lactate [2]. Carbon skeleton from glucose can also be used in biosynthetic pathways, namely pentoses for nucleic acids.

Glutamine is described as the most important source of nitrogen in tumours; the mutual interactions between glucose and glutamine in Ehrlich cells is under discussion [1, 3–5].

The aim of the present study is to determine the short-term effect of glutamine on glucose utilization and the incorporation of carbon skeleton derived from glucose into nucleic acids.

Materials and Methods

O-(U-¹⁴C)Glucose (10⁴ GBq/mmol), O-(1-¹⁴C)glucose (2·27 GBq/mmol), α-(6-¹⁴C)glucose (2·08 GBq/mmol) and (methyl-³H)-thymidine (185 GBq/mmol) were supplied by Amersham International, Amersham, England. Enzymes were bought to Boehringer, Mannheim, Germany. All other reagents were from Merck, Darmstadt, Germany. Hyperdiploid Lettré Ehrlich ascites tumour cells were grown and harvested as described elsewhere [6]. Cells were suspended in phosphate buffered saline, pH 7·4. Cellular density was adjusted to 120×10⁶ cells/ml. The cells were preincubated at 37°C in a 50 ml flask under an atmosphere of 95% O₂ and 5% CO₂ for 15 min. The suspension was then diluted to 60×10⁶ cells/ml in flasks containing the incubation medium and substrates. The incubations were carried out at 37°C in a Grant metabolic incubator with continuous shaking. Glycolytic intermediates and lactate were determined enzymatically [7–10]. The relative rate of DNA synthesis was measured by incorporation of (methyl-³H)thymidine into acid insoluble material. In order to measure the incorporation of glucose-carbon into nucleic acids, incubations were carried out in the presence of 5 mM glucose, or 5 mM glucose plus 0·5 mM glutamine, added with O-(U-¹⁴C)-glucose. Incubations were stopped and radioactivity in RNA and DNA was measured according to Kennell [11]. The glucose consumed in the pentose phosphate pathway was determined by the method of Katz et al [12].

Results and Discussion

Glucose slightly increased the rate of DNA synthesis measured by incorporation of tritiated thymidine, as compared with cells incubated in the absence of glucose (results not shown). Table I shows the incorporation into nucleic acids (DNA+RNA) of labelled glucose-carbone skeleton, and the relative values of the total glucose consumed, after 15 min of incubation when about 60% of glucose was consumed by the cells, and after 60 min when glucose had been totally exhausted. This incorporation significantly raised in incubations added with 0·5 mM glutamine. It is noteworthy that radioactivity from glucose carbon skeleton was incorporated in both DNA and RNA molecules.
Table I. Short-term incorporation of $\alpha$-($U^{14}C$)-glucose carbon skeleton into nucleic acids in Ehrlich ascites tumour cells.

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Substrates added</th>
<th>15 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (5 mM)</td>
<td>4475±236 (1.30%)</td>
<td>11890±665 (1.92%)</td>
<td></td>
</tr>
<tr>
<td>Glucose (5 mM) + glutamine (0.5 mM)</td>
<td>5560±359 (1.50%)</td>
<td>13942±428 (2.25%)</td>
<td></td>
</tr>
</tbody>
</table>

Data are given as dpm and are mean ± standard error of mean of 5 separate experiments. In brackets, the percentages of consumed glucose-carbon incorporated into nucleic acids (DNA+RNA).

Significant \(P<0.01\), according to a Student’s t test.

After batch incubations in the presence of only 5 mM glucose, as well as in the presence of glucose added with 0.5 mM glutamine. In fact, after the separation, 1/3 of the total radioactivity was found in the DNA fraction, the other two thirds being incorporated in the RNA fraction. These results support the fact that in short-term experiments both processes, DNA replication and transcription in RNA, are active and accelerated by physiological concentrations of glutamine. These results are in good agreement with those obtained for glucose consumed in the oxidative pentose phosphate pathway; indeed, from the ratio $^{14}$CO$_2$ evolved from (1$^{14}$C) glucose and (6$^{14}$C) glucose, it was calculated that only 0.45% and 0.97% of the total glucose consumed at 15 and 60 min respectively was utilized in this pathway. The contribution of the non-oxidative pentose phosphate pathway to the total pentoses from glucose incorporated to nucleic acid was approximately 0.85% and 0.95% after 15 and 60 min, respectively.

As Table II shows, besides lactate production being slightly enhanced, 0.5 mM glutamine did not modify the levels of intermediates of the glycolytic process. Medina et al [1] report that glutamine changes neither glucose consumption nor glucose oxidation.

The results presented reveal that in short-term experiments glucose is utilized in the nucleic acid synthesis in Ehrlich ascites tumour. Glutamine slightly but significantly increases the rate of incorporation of glucose-carbon into nucleic acids catabolized through the pentose phosphate pathways, mainly in the RNA fraction. Nevertheless, the glucose-carbon skeletons incorporated into nucleic acids are much fewer as compared with those dissipated to lactate [13]. These data reinforce the previous statement on the preferential utilization of glucose over other metabolic substrates by tumour cells [1, 14].

Acknowledgments
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Table II. Glycolytic intermediates produced by the Ehrlich cells after 15 min of incubation in the presence of glucose plus glutamine.

<table>
<thead>
<tr>
<th>Intermediates</th>
<th>Buffer</th>
<th>Glucose</th>
<th>Glucose + glutamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-6-phosphate</td>
<td>0.02±0.01(4)</td>
<td>2.42±0.18(5)</td>
<td>2.38±0.18(5)</td>
</tr>
<tr>
<td>Fructose-6-phosphate</td>
<td></td>
<td>0.69±0.05(5)</td>
<td>0.69±0.05(5)</td>
</tr>
<tr>
<td>Fructose-1,6-bisphosphate</td>
<td>0.03±0.01(3)</td>
<td>3.83±0.44(3)</td>
<td>3.81±0.39(3)</td>
</tr>
<tr>
<td>Dihydroxyacetone-3-phosphate</td>
<td>0.16±0.05(4)</td>
<td>1.17±0.10(5)</td>
<td>1.08±0.12(5)</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate</td>
<td>0.06±0.04(5)</td>
<td>1.08±0.04(5)</td>
<td>1.06±0.02(5)</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.20±0.03(5)</td>
<td>3.20±0.20(5)</td>
<td>2.20±0.30(5)</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.90±0.50(5)</td>
<td>52.30±4.20(6)</td>
<td>66.80±2.30(7)*</td>
</tr>
</tbody>
</table>

Glycolytic intermediates were enzymatically measured at 0 and 15 min in cells incubated with no added substrate, with 5 mM glucose or with 5 mM glucose and 0.5 mM glutamine. Data are given as nmol / 10$^6$ cells and are mean ± SEM with the number of separate experiments given in brackets. The values at time zero were: glucose-6-phosphate, 0.07; fructose-1,6-bisphosphate, 0.06; dihydroxyacetone-3-phosphate, 0.09; pyruvate, 0.50; and lactate, 6 nmol / 10$^6$ cells; fructose-6-phosphate and glyceraldehyde-3-phosphate were indetectable.

* Significant \(P<0.01\) versus cells incubated with only glucose, according to a Student’s t test.
References