Respiratory Activity of Mitochondria Isolated from a Transplantable Islet-Cell Tumor and from the Liver of Tumor-bearing Hamsters

Milton D. Gross, Albert J. Whitty, and Piero P. Foà
Department of Research, Sinai Hospital of Detroit, Detroit, Michigan 48235

SUMMARY

This paper describes the respiration of mitochondria isolated from a transplantable pancreatic islet-cell tumor of the hamster and the rate of oxidative phosphorylation in hepatic mitochondria of tumor-bearing animals. The oxygen uptake and, in many cases, the adenosine diphosphate to oxygen (ADP/O) and respiratory control ratios of tumor and liver mitochondria in the presence of a variety of substrates were relatively high, reflecting the high metabolic activity of the tumor and, possibly, the effect of tumor-induced hyperinsulinism.

INTRODUCTION

Kirkman (13) with Algard (14) described a transplantable pancreatic islet-cell tumor that causes hyperinsulinism and fasting hypoglycemia (18) in the host. The tumor has many interesting biological characteristics (21), including a relatively high content of oxidative enzymes (8), such as NAD diaphorase; NADPH diaphorase; lactic, succinic, and glucose 6-phosphate dehydrogenases; and cytochrome oxidase. The tumor contains abundant mitochondria, but these are often deformed and generally of variable shapes (17, 20). Possibly, these mitochondrial abnormalities are the cause of the high rate of anaerobic metabolism characteristic of this tumor (20). It has been suggested also that the high rate of glycolysis in the tumor may be due to its relatively high content of serotonin and monoamine oxidase, and, consequently, of the carbonyl derivative of serotonin (15, 19), a substance that reduces the efficiency of oxidative phosphorylation.

This paper describes the results of experiments which indicate that, in the presence of several substrates, oxygen uptake, ADP/O ratio, and RCR of the tumor mitochondria were relatively high and that the presence of the tumor in the intact host animal increased oxygen uptake by the liver mitochondria.

MATERIALS AND METHODS

Adult female golden hamsters, weighing 80 to 100 g, were kept in individual cages and fed Purina laboratory chow ad libitum. The tumor was transplanted by means of s.c. injections of selected fragments of nonnecrotic tumor tissue, freshly excised from tumor-bearing but otherwise healthy animals and suspended in sterile 0.9% NaCl solution. Blood glucose was determined enzymatically with the Glucostat assay (Worthington Biochemical Corp., Freehold, N. J.). On or about the 5th week after transplantation, when the tumor nodules were palpable, the animals were sacrificed by cervical dislocation and the mitochondria were isolated from the liver and the tumor, as follows (4, 5).

Liver and tumor tissue, cleansed of connective tissue and of any necrotic material, were blotted, weighed, minced, and washed in ice-cold 0.18 M KCl until blood was no longer visible in the washings. From 10 to 12 g of tissue were extracted with a solution consisting of 0.014 M Tris, 0.25 M sucrose, 0.18 M KCl, 10 mM EDTA, and 1% BSA, pH 7.5. After extraction the tissue was homogenized with a rheostatically controlled, motor-driven Teflon glass homogenizer (Kontes Glass Co., No. F24R, Franklin Park, Ill.) in 9 volumes of the same buffer. Nuclei, unbroken cells, and erythrocytes were removed by centrifugation at 600 X g for 10 min. The supernatant was centrifuged at 10,000 X g for 10 min, the pellet thus obtained was resuspended in one-half the original volume of buffer, with a hand-operated Teflon glass homogenizer (size 23) and recentrifuged at 5,900 X g for 10 min. Finally, the mitochondrial pellet was suspended in 0.25 M sucrose solution with a hand-operated Teflon Size AA glass homogenizer. Mitochondrial protein was determined by a biuret procedure (5, 7) after solubilization of the particles with sodium deoxycholate. For this purpose, 1.5 ml of biuret reagent and 0.05 ml of mitochondrial suspension were mixed in a test tube. Water and sodium deoxycholate (5 mg) were added to a total volume of 3 ml, and the contents were mixed by gentle inversion to avoid foaming. The pH was 8.0. Reagent blanks and tubes containing standard solutions of crystalline BSA, instead of mitochondrial suspension, also were prepared. The tubes were placed in a boiling water bath for 30 sec and cooled for 1 min in ice; the A\textsubscript{405nm} was determined immediately. BSA solutions were standardized according to the method of Kaziro et al. (11). Oxygen consumption was determined polarographically with a Clark Model 5331 electrode (Yellow Springs Instrument Company, Yellow Springs, Ohio), in a 1.5 ml water-jacketed glass cell maintained...
at 25°. Signals from the Clark electrode were monitored with Heath Model EUW-20A Servo recorder (Heath Company, Benton Harbor, Mich.) as described by Estabrook (6). The concentration of dissolved oxygen was assumed to be 240 μM at 25°. The mitochondria were suspended in a medium of 0.15 M sucrose, 0.025 M Tris-HCl, and 0.006 M K2HPO4, pH 7.5. The substrates pyruvate, glutamate, and succinate (highest purity) were obtained from Fisher Scientific Company, Detroit, Mich. Malate, also of highest purity, was from J. T. Baker Chemical Company, Phillipsburg, N. J., supplied by Will Corporation of Ann Arbor, Mich. Pyruvate, malate, glutamate, and palmitoylcarnitine were in buffered solutions and at pH 7.4. Succinate solution was also buffered and at pH 7.5. Substrates and other reagents were added to the reaction cuvet through thin polyethylene tubes fitted to microliter syringes.

RESULTS

The blood glucose of tumor-bearing animals, measured at the time of sacrifice, was markedly lower than that of the controls (Table 1). Table 2 shows the rates of substrate oxidation by liver mitochondria of control and tumor-bearing animals in the presence of ADP (State 3) and after exhaustion of the ADP supply (State 4). Significant differences were observed between the respiratory rates of mitochondrial preparations from control and tumor-bearing animals in States 3 and 4 when palmitoylcarnitine and succinate, but not glutamate and pyruvate, were used as substrates.

Table 1
Control and recipient tumor hamsters

<table>
<thead>
<tr>
<th>Blood glucose (mg/100 ml)</th>
<th>No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>112.2 ± 6.96a</td>
</tr>
<tr>
<td>Recipient</td>
<td>68.88 ± 9.09</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.E.

Table 2
Respiratory activities of liver mitochondria from control and recipient tumor-bearing hamsters

The rates of oxidation were determined polarographically as described in “Materials and Methods.” Concentrations of reagents were (mM): pyruvate, 16.7; L-malate, 1.67; glutamate, 5.58; succinate, 16.7; palmitoyl-L-carnitine, 1.58. Numbers in parentheses indicate probability values (Student’s t test vs. control values) and degrees of freedom. Only those p values that approach significance are included.

<table>
<thead>
<tr>
<th>Respiratory rate (nanoatoms O2/min/mg protein)</th>
<th>State 3</th>
<th>State 4</th>
<th>RCR</th>
<th>ADP/O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate-malate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>21.58 ± 1.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.08 ± 0.75</td>
<td>3.41 ± 0.49</td>
<td>2.47 ± 0.089</td>
</tr>
<tr>
<td>Recipient</td>
<td>25.16 ± 1.51</td>
<td>8.54 ± 0.81</td>
<td>3.16 ± 0.28</td>
<td>2.52 ± 0.05</td>
</tr>
<tr>
<td>Glutamate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>26.30 ± 0.88</td>
<td>6.97 ± 0.50</td>
<td>4.04 ± 0.33</td>
<td>2.50 ± 0.06</td>
</tr>
<tr>
<td>Recipient</td>
<td>36.52 ± 2.18</td>
<td>8.64 ± 0.69</td>
<td>4.36 ± 2.57</td>
<td>2.48 ± 0.06</td>
</tr>
<tr>
<td>Palmitoylcarnitine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>24.82 ± 0.97</td>
<td>5.96 ± 0.44</td>
<td>4.52 ± 0.40</td>
<td>2.44 ± 0.05</td>
</tr>
<tr>
<td>Recipient</td>
<td>35.69 ± 1.95</td>
<td>9.89 ± 0.72</td>
<td>3.87 ± 0.36</td>
<td>2.25 ± 0.04</td>
</tr>
<tr>
<td>(&lt;0.001;15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>31.31 ± 1.22</td>
<td>17.10 ± 1.25</td>
<td>2.01 ± 0.12</td>
<td>1.42 ± 0.89</td>
</tr>
<tr>
<td>Recipient</td>
<td>47.20 ± 4.32</td>
<td>22.00 ± 2.12</td>
<td>2.15 ± 0.12</td>
<td>1.21 ± 0.07</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.E.

DISCUSSION

The tumor used in our experiments, in spite of mitochondrial abnormalities and the high content of glycolytic enzymes, has high rates of oxygen uptake, ADP/O, and RCR’s comparable not only to those of other tumors (1, 2, 23) but also to those of normal liver mitochondria. For example, P/O ratios for mitochondria obtained from a transplantable mouse...
Mitochondria

Respiratory rate

nanoatoms O$_2$/min/mg mitochondrial protein

<table>
<thead>
<tr>
<th>Mitochondria</th>
<th>State 3</th>
<th>State 4</th>
<th>RCR</th>
<th>ADP/O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor</td>
<td>31.64 ± 3.84</td>
<td>12.39 ± 1.56</td>
<td>2.75 ± 0.32</td>
<td>2.24 ± 0.11</td>
</tr>
<tr>
<td>Normal liver</td>
<td>26.30 ± 0.88</td>
<td>6.97 ± 0.50</td>
<td>0.01; 15</td>
<td>0.05; 15</td>
</tr>
</tbody>
</table>

Pyruvate-malate

<table>
<thead>
<tr>
<th>Mitochondria</th>
<th>State 3</th>
<th>State 4</th>
<th>RCR</th>
<th>ADP/O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>44.37 ± 5.61</td>
<td>16.01 ± 1.98</td>
<td>2.86 ± 0.25</td>
<td>2.41 ± 0.14</td>
</tr>
<tr>
<td>Normal liver</td>
<td>21.58 ± 1.15</td>
<td>7.08 ± 0.75</td>
<td>0.01; 13</td>
<td>0.001; 13</td>
</tr>
</tbody>
</table>

Palmitoyl carnitine

<table>
<thead>
<tr>
<th>Mitochondria</th>
<th>State 3</th>
<th>State 4</th>
<th>RCR</th>
<th>ADP/O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>42.55 ± 5.07</td>
<td>15.24 ± 1.65</td>
<td>2.86 ± 0.29</td>
<td>2.10 ± 0.16</td>
</tr>
<tr>
<td>Normal liver</td>
<td>24.82 ± 0.97</td>
<td>5.96 ± 0.44</td>
<td>0.01; 14</td>
<td>0.01; 14</td>
</tr>
</tbody>
</table>

Succinate

<table>
<thead>
<tr>
<th>Mitochondria</th>
<th>State 3</th>
<th>State 4</th>
<th>RCR</th>
<th>ADP/O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>170.84 ± 11.84</td>
<td>94.44 ± 7.57</td>
<td>1.82 ± 0.03</td>
<td>1.14 ± 0.09</td>
</tr>
<tr>
<td>Normal liver</td>
<td>31.31 ± 1.22</td>
<td>17.10 ± 1.25</td>
<td>0.001; 12</td>
<td>0.001; 12</td>
</tr>
</tbody>
</table>

Control Liver Mit. 8.52 mg

1.5 ml Medium

Glutamate 5.58 mM

212.6 µM ADP

6.27

10.27

212.6 µM ADP

Liver Mit. - Tumor Anim. 5.07 mg.

1.5 ml Medium

Glutamate 5.58 mM

212.6 µM ADP

6.27

10.27

212.6 µM ADP

36 nates O

-60 sec

Chart 1. Oxygen electrode tracings of glutamate oxidation in the presence of liver mitochondria (Mit.) from control hamsters. The numbers above the tracing indicate the rate of oxygen reduction in nanoatoms O$_2$/min/mg mitochondrial protein. Average values: ADP/O, 2.45; RCR, 3.85; glutamate (5.58 mM). Time course is from left to right.

hepatoma were 2.22 and 1.55 in the presence of α-ketoglutarate and succinate, respectively, while the corresponding ratios of mitochondria from normal hepatic tissue were 2.42 and 1.58 (12). The question arose as to the need for exogenous Mg$^{2+}$. It should be realized that Mg$^{2+}$ has been used widely as a cofactor for an ATP-trapping system, usually hexokinase-glucose. In the polarographic assay, such a trapping system is not used. In addition, liver mitochondrial preparations contain about 5 to 20 nmoles Mg$^{2+}$ per mg protein bound to the mitochondrial structure; this amount appears to be sufficient in concentration and internal localization to support the phosphorylation of ADP. It has been determined that the addition of Mg$^{2+}$ to such respiring mitochondria...
preparations is unnecessary. When one further considers the purity and stability of the liver mitochondrial preparations utilizing sucrose to prevent aggregation, the high respiratory control of between 3 and 5 clearly indicates that little if any extramitochondrial material aggregated with the mitochondria. Our mitochondrial preparations have been observed to provide high RCR's for at least 5 hr after the final pellet is suspended and maintained in the ice bath. Attention was given to the variable concentrations of protein in the different preparations. It has been determined experimentally in the type of polarographic assay used that rates of substrate oxidation are linear between 1 and 10 mg protein per cuvet. Above 10 mg of protein, linearity ceases and the rate of oxygen diffusion across the 1-mil Teflon membrane becomes limiting. One can extend the linearity of the curve by using 0.5-mil Teflon membranes. Our values for protein concentration all fall within the 1- to 10-mg range. When one considers the higher substrate utilization by tumor mitochondria in State 3 respiration, the speculation is that the tumor mitochondria may simply be more "open" to the substrate, especially succinate. The higher rates of the tumor mitochondria may be a reflection of a greater number of respiratory assemblies per mg protein. These oxidative reactions may supply the energy not only for the metabolic requirement of a rapidly growing tissue but also for the synthesis of insulin by the tumor cells. A similar requirement has been demonstrated for normal pancreatic insulin release (3). On the other hand, the tumor-induced hyperinsulinism may contribute to the accelerated rate of oxygen uptake by the liver mitochondria of tumor-bearing animals.

The effect of insulin on mitochondrial oxidative phosphorylation prepared from diabetic animals has been reported by several investigators. Parks et al. (16) found no difference in the uptake of inorganic phosphate and oxygen consumption...
between mitochondria from normal and those from alloxan-diabetic rats and no effect of insulin added in vitro. Vester and Stadie (22) confirmed these results but found that mitochondria from depancreatized rats had low P/O ratios which were not affected by insulin in vitro. However, these ratios were restored to normal after 3 days of insulin treatment in vivo. Hall et al. (10) have shown a clear-cut defect in oxidative phosphorylation in liver mitochondria, prepared from alloxan-diabetic rats, which is sensitive to insulin both in vivo and in vitro. Gross et al. (9) noted a respiratory defect in skeletal muscle of rats with chronic diabetes induced by streptozotocin, under control conditions, and after the addition of ADP. These results were interpreted to indicate that lesions in the energy-releasing and conserving reactions of skeletal muscle mitochondria were not primary but secondary expressions of tissue degeneration that accompanies the untreated diabetic state. Our study indicates that chronic hyperinsulinemia accelerates the respiratory activity of hepatic mitochondria.

ACKNOWLEDGMENTS

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REFERENCES

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