A Study of the Effect of Amytal on the Respiration of Rat Liver Slices

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It has been reported that rat liver slices, incubated for 1 h in a Krebs-Ringer solution containing glucose, exhibit an oxygen uptake which is resistant to amytyl (5-ethyl-5-isooamyl-barbituric acid) to an extent of about 40%. Closer examination of this amytyl-resistant respiration has now revealed that it is characterized by a declining initial phase, lasting about 20-30 min, during which its rate drops from about 60 to 20% of the control rate, followed by a second stationary phase where the respiration remains constant at about 20% of the control rate over a period of at least 1 h. Omission of glucose and/or addition of α-ketoglutarate, malate, glutamate, lactate and glycerol-1-phosphate increased the respiration with and without amytyl (Table 1). Addition of the glycolytic inhibitors, iodoacetate or fluoride, both of which strongly depressed the control rate, had little effect on the level of the amytyl-resistant respiration. Addition of succinate caused, in agreement with earlier literature, about a ten fold increase of the respiratory rate, and this respiration was entirely amytyl-insensitive. This finding is consistent with the concept that succinate is oxidized by an amytyl-insensitive oxidase.

Added DPN or TPN caused about an equal, 50-100% stimulation of the control respiratory rate both in the absence of added substrate and in the presence of added glucose or the previously mentioned pyridine nucleotide-linked substrates. The two pyridine nucleotides differed, however, with respect to their effect on the amytyl-resistant respiration, this being stimulated more by TPN than by DPN. Moreover DPN and TPN added together stimulated the amytyl-resistant respiration by more than the sum of the individual stimulations. These effects were apparent only on the stationary, and not the initial, phase of the amytyl-resistant respiration. It would therefore seem that while the initial phase of the amytyl resistant respiration probably originates from some endogenous substrate which is not continuously formed during incubation, the constant respiration phase is due to substrates which are continuously available, and part of which, at least, appear to be oxidized by way of TPN. The glucose-6-phosphate dehydrogenase shunt would seem to be a likely reaction of this type. That added DPN, in combination with TPN, further increases the amytyl-resistant respiratory rate, may be due to the stimulation of a pyridine nucleotide transhydrogenase mechanism, conceivably through the mediation of lactic dehydrogenase.

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Table 1. Effect of added substrates and pyridine nucleotides on the amytyl-resistant respiration of rat liver slices, 80 to 120 mg of liver slices (20 mg with succinate) were incubated for 1 h at 37°C in Krebs-Ringer-phosphate solution (half Ca++ conc.) with 100% O2 as gas phase. Final volume, 1 ml; final concentrations of the additions were: glucose, α-ketoglutarate and malate, 0.025 M; glycerol-1-phosphate, lactate, glutamate and succinate, 0.05 M; amytyl, 0.002 M.

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Additions</th>
<th>μatoms oxygen/60 min./100 mg wet weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>without amytyl</td>
</tr>
<tr>
<td>1</td>
<td>none</td>
<td>4.57</td>
</tr>
<tr>
<td></td>
<td>glucose</td>
<td>4.11</td>
</tr>
<tr>
<td>2</td>
<td>none</td>
<td>4.51</td>
</tr>
<tr>
<td></td>
<td>α-ketoglutarate</td>
<td>6.12</td>
</tr>
<tr>
<td></td>
<td>malate</td>
<td>5.36</td>
</tr>
<tr>
<td>3</td>
<td>none</td>
<td>4.21</td>
</tr>
<tr>
<td></td>
<td>glutamate</td>
<td>7.15</td>
</tr>
<tr>
<td></td>
<td>succinate</td>
<td>55.4</td>
</tr>
<tr>
<td>4</td>
<td>none</td>
<td>6.07</td>
</tr>
<tr>
<td></td>
<td>lactate</td>
<td>8.90</td>
</tr>
<tr>
<td></td>
<td>glycerol-1-phosph.</td>
<td>7.37</td>
</tr>
<tr>
<td>5</td>
<td>glucose</td>
<td>3.98</td>
</tr>
<tr>
<td></td>
<td>glucose + DPN</td>
<td>5.58</td>
</tr>
<tr>
<td></td>
<td>glucose + TPN</td>
<td>5.65</td>
</tr>
<tr>
<td></td>
<td>gluc. + DPN + TPN</td>
<td>7.85</td>
</tr>
</tbody>
</table>

Table 2. Dicoumarol-sensitive respiration of rat liver slices. Conditions as in Table 1. Final concentrations of the additions were: glucose, 0.05 M; iodoacetate, 0.2 mM; amyyl 2 mM; vitamin K₃-bisulfite, 0.005 mM; citrate, 0.025 M; TPN, 0.001 M; dicoumarol, 0.005 mM. The system was preincubated for 20 min in the presence of all additions except amyyl. The values give the respiration obtained during 48 min following the addition of amyyl.

<table>
<thead>
<tr>
<th>Additions</th>
<th>μatoms oxygen/100 mg wet weight</th>
<th>without dicoumarol</th>
<th>with dicoumarol</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>1.79</td>
<td>2.43</td>
<td>-39</td>
<td></td>
</tr>
<tr>
<td>vitamin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K₃-bisulfite</td>
<td>1.31</td>
<td>1.53</td>
<td>-19</td>
<td></td>
</tr>
<tr>
<td>citrate</td>
<td>2.46</td>
<td>1.72</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>citrate + vit.</td>
<td>4.06</td>
<td>2.46</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>K₃-bis.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>citrate + vit. + TPN 7.88</td>
<td>4.61</td>
<td>2.46</td>
<td>39</td>
<td></td>
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</tbody>
</table>

The presence of iodoacetate in order to exhaust glycolytic intermediates, and subsequent incubation with added citrate, resulted in an increased amyyl-resistant respiration which now was inhibited by 5 × 10⁻⁴ M dicoumarol to about 30%. Moreover, addition of vitamin K₃ or of vitamin K₃ and TPN both of which further increased the amyyl-resistant respiratory rate, also increased its dicoumarol sensitivity in an absolute as well as relative fashion. These findings are preliminary interpreted to indicate that the DT diaphorase apparently takes no major part in the normal respiration of the liver cell, but may act as an alternative pathway of TPNH (and DPNH) oxidation, which can be made manifest experimentally by suppressing more efficient pathways. The main interest of these findings may lie in the fact that a dicoumarol-sensitive respiration, i.e. a DT diaphorase reaction, has been demonstrated in these conditions to function in the intact liver cell even without supplementation with vitamin K₃.

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