Metabolic Changes in the Lungs after Ischaemia

F. M. ENGELBRECHT, I. J. EDWARDS, D. P. DE BEER

SUMMARY

The effects of variable periods of ischaemia on the isolated lungs of rats and rabbits, stored for up to 6 hours at 4°C, 21°C and 37°C under standardized conditions, were investigated in vitro in terms of oxygen consumption, the rate of 1-14C-leucine incorporation into soluble proteins, and 1-14C-palmitate incorporation into total phospholipids and lipid fractions.

The endogenous oxygen uptake of rat lung slices in an air phase, from tissues stored at 4°C and 21°C under ischaemic conditions for 6 hours and at 37°C for 4 hours, was significantly different from the control values. The oxygen uptake of lungs from animals anaesthetized with pentobarbitone prior to exsanguination and stored for only 2 hours at 37°C differed significantly from control values.

Judged by the rate of incorporation of radiolabelled leucine into soluble proteins and that of palmitate into total lipids and phospholipids of lungs after storage for increasing periods at 4°C and 37°C, significant differences were already found after 1½ hours. From this observation it would appear that these parameters are very sensitive indicators for assessing irreversible lung damage due to ischaemia.


The literature on experimental lung transplantation shows that many attempts have been made in several species without much success, in spite of modern surgical technology. One reason for the lack of success may be irreversible metabolic damage to the lungs as a result of ischaemia. The 'survival-after-implantation' technique has been used to evaluate the methods for lung preservation, but this approach appears to be laborious and expensive.

We believe that further research into metabolic changes in the isolated lung could provide useful information on methods for preservation, and might even solve some of the problems involved in successful lung transplantation.

The effects of ischaemia on the structure, dynamic properties and gas exchange of the lung have been studied extensively. Von Wichert attempted to relate the duration of ischaemia to metabolic changes in isolated normothermic rabbit lungs. They found a gradual decrease in glucose and adenosine triphosphate (ATP) content over a period of 3 hours accompanied by an increase in lactate concentration, although the total amount of phospholipids was not affected. Shimada et al. studied the effects of ischaemia on isolated dog lungs, kept in vitro at 0° to 4°C. They demonstrated that the rates of oxidation of 1-14C-glucose and 1-14C-acetate to 14CO2 decreased significantly after ischaemia lasting 2 and 4 hours respectively. Total glucose consumption was reduced by about 30% after 30 minutes and remained constant at that level for up to 4 hours of ischaemia.

In view of the problems encountered with lung transplantsations, the present research was initiated to determine the degree of lung damage due to ischaemia. Lungs of rats and rabbits were, after isolation, stored at various temperatures and under standardized conditions for different periods of time, whenupon the rate of (i) oxygen utilization; (ii) incorporation of 1-14C-leucine into soluble proteins; and (iii) incorporation of 1-14C-palmitate into phospholipids of the lung was measured.

MATERIALS AND METHODS

New Zealand White rabbits (1.5 - 2.0 kg) and Long-Evans rats (180 - 200 g) were used. The rats were either sacrificed by decapitation or exsanguination by severing the abdominal aorta under sodium pentobarbitone anaesthesia (10 mg/100 g intraperitoneally) (Nembutal Veterinary: Abbott Laboratories). The rabbits were anaesthetized with a sublethal dose of 2.5% thiopental sodium (18 mg/kg intravenously). The thorax was opened quickly and the lungs were perfused in situ via the pulmonary artery with 15 ml (rats) and 50 ml (rabbits) isotonic saline at 4°C. The rat lungs were ventilated during perfusion. The lungs were then quickly removed and dissected free of large airways and blood vessels. Lungs with any macroscopic signs of disease were discarded.

The isolated lungs were cut into sections of ± 300 mg each. For non-ischaemic control experiments, one section was immediately chopped into 0.7 mm slices (McIlwain tissue slicer) and incubated at 37°C (see individual experiments). The other sections were stored for specific time intervals at 4°C, 21°C and 37°C in 10 ml glass containers lined with filter paper and moistened with buffered medium. At indicated intervals, the stored tissue was removed from the containers and cut into 0.7 mm slices to be used in the different incubation experiments. As far as possible, one of each pair of lungs was used for the experiment while the other provided the control.

Oxygen Consumption

The rate of oxygen consumption of normal and ischaemic lung slices, stored for up to 6 hours, was determined over 1 hour according to the direct Warburg technique using a Braun's Warburg apparatus model...
The values obtained with ischaemic lungs of anae­thesitized rats clearly demonstrated the additional dele­terious effect of pentobarbitone on the rate of oxygen uptake with time (Fig. 1).
In Fig. 2 the oxygen uptakes of ischaemic rat and rabbit lung slices in a 100% O₂ phase with 10 mM glucose as substrate are compared. The oxygen uptake of rat lung slices stored at 4°C and 21°C for up to 4½ hours was not significantly different under these conditions from the control values. However, the oxygen uptake of rabbit lung, kept under similar conditions, decreased significantly after 3 hours of ischaemia. When ischaemic lungs of both species were stored at 37°C, a marked decline in oxygen uptake occurred, which was already significantly different from control values after 1¾ hours (P<0,01) and became even more significant up to 4½ hours after storage.

In Fig. 3A and B, the effects of ischaemia after 1½ and 3 hours at 4°C and 37°C on 1-¹C-leucine and 1-¹C-palmitate incorporation into lung proteins and lipids respectively are recorded. The mean rate of 1-¹C-leucine incorporation into the soluble proteins of control lung slices over 2 hours amounted to 18835 ± 316 cpm/mg isolated protein. After storage of ischaemic lung for 1½ hours at 4°C, the rate of 1-¹C-leucine incorporation decreased significantly (P<0,01), while in lung tissue kept at 37°C the reduction was even more dramatic (Fig. 3A).

The amount of soluble protein isolated from ischaemic lung tissue stored at 4°C and 37°C (Table I) and thereafter incubated for 2 hours gradually decreased with the duration of ischaemia and was significantly different from the control value obtained from the same lung tissue without storage.

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**TABLE I. SOLUBLE PROTEIN ISOLATED FROM ISCHAEMIC LUNG SLICES PRESERVED AT 4°C AND 37°C AND AFTER 2 HOURS' INCUBATION**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Temp. (°C)</th>
<th>mg protein/100 mg wet tissue</th>
<th>% of control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>2,31 ± 0,11</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ischaemic for 1½ h</td>
<td>4</td>
<td>2,03 ± 0,20</td>
<td>88</td>
<td>0,01</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>1,41 ± 0,14</td>
<td>61</td>
<td>0,001</td>
</tr>
<tr>
<td>Ischaemic for 3 h</td>
<td>4</td>
<td>1,93 ± 0,15</td>
<td>84</td>
<td>0,01</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>1,30 ± 0,38</td>
<td>56</td>
<td>0,001</td>
</tr>
</tbody>
</table>
Incorporation of 1-¹³C-palmitate into the total extractable lipids of control lung slices over 2 hours amounted to 44,890 ± 388 cpm/300 mg tissue. The rate of incorporation into the lipids of ischaemic lung tissue stored at 4°C and 37°C for 1 1/2 and 3 hours was significantly lower than the control value at both temperatures and time intervals (Fig. 3B).

The results recorded in Table II also demonstrated that the rate of 1-¹³C-palmitate incorporation into the total phospholipid fraction as well as the dipalmitoyl phosphatidyl choline fraction of the phospholipids after storage at 37°C was already markedly reduced after 1 1/2 hours; this tendency progressed with time. By contrast, at 4°C the rate of 1-¹³C-palmitate incorporation into the ischaemic lung phospholipids remained normal up to 3 hours. This also applies to the amount of labelled palmitate in free fatty acid and neutral lipid fractions from ischaemic lung stored at 4°C and 37°C for up to 3 hours.

**DISCUSSION**

Using the rate of oxygen consumption as an index of the degree of metabolic damage induced by ischaemia at different temperatures for various periods, our findings clearly demonstrate that ischaemia at temperatures of 4°C and 21°C and lasting 4 hours did not suppress aerobic metabolism in the lung as in other tissues.

**TABLE II. ¹³C-PALMITATE INCORPORATED INTO TOTAL PHOSPHOLIPIDS, PHOSPHATIDYL CHOLINE, FATTY ACID AND NEUTRAL LIPID FRACTIONS (EXPRESSED AS CPM PER 1 mg TOTAL LIPID EXTRACTED)**

<table>
<thead>
<tr>
<th>Ischaemia</th>
<th>Control</th>
<th>1 1/2 h</th>
<th>3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phospholipids</td>
<td>1,703 ± 221</td>
<td>1,566 ± 290</td>
<td>721 ± 252*</td>
</tr>
<tr>
<td>Phosphatidyl choline</td>
<td>1,299 ± 147</td>
<td>1,205 ± 205</td>
<td>559 ± 195*</td>
</tr>
<tr>
<td>Fatty acid and neutral lipids</td>
<td>805 ± 169</td>
<td>718 ± 262</td>
<td>791 ± 355</td>
</tr>
</tbody>
</table>

*Indicates significant deviation from control.
metabolism significantly. However, ischaemia at 37°C for 4 hours and longer depressed oxygen uptake markedly and progressively. Because the ischaemic slices used in the first experiment (Fig. 1) were not challenged with substrate after ischaemic storage, the observed rate of oxygen uptake might not reflect their maximal oxygen utilization. However, normal lung tissue utilizes oxygen at a very constant rate for up to 6 hours and longer without exogenous substrate. It is known that the amount of lung glycogen is limited and the lung must therefore either metabolize fatty acids or amino acids. When exogenous glucose was added (Fig. 2) the rate of oxygen utilization was slightly, but not significantly, higher. The reduction in oxygen uptake of ischaemic rat lung tissue kept at 4°C and 21°C for up to 4 hours was never significantly different from control values, irrespective of whether glucose was available as substrate or not. However, all tissue stored at 37°C suffered significant damage after 4 hours of ischaemia, both in the presence and in the absence of glucose.

It has been shown that during ischaemia a progressive decrease in ATP synthesis in lung tissue occurs, while glycolysis is increased. The resulting pyruvate is increasingly converted to lactate under anaerobic conditions. The more glucose is metabolized anaerobically, the higher the lactate concentration becomes and the lower the intracellular pH. The longer the ischaemic period and the higher the temperature during ischaemia, the greater will be the reduction in aerobic metabolism. However, it would appear from our findings that ischaemic lung tissue could be kept at 4°C and 21°C for up to 4 hours without incurring serious irreversible damage. The rate of oxygen uptake of these tissues is so close to normal that some of the changes may be reversed if the oxygen supply is restored and ATP synthesis recommences. However, many lung transplants have been unsuccessful, some even after relative short periods of ischaemia. This might indicate that, although short periods of ischaemia induce only a small reduction in aerobic metabolism, some other changes might be irreversible. The sensitivity of lung tissue to damage is demonstrated by the significant suppressive effect of sodium pentobarbitone anaesthesia on the oxygen utilization of ischaemic lungs at 37°C. Another important finding is that rabbit lung is more sensitive to ischaemic damage at temperatures between 4°C and 21°C than is rat lung. This might be due to the high sensitivity of rabbit lung to oxygen toxicity in a 100% oxygen phase.

A similar difference may exist among other species.

In the evaluation of protein synthesis by ischaemic lung tissues, a significant deviation from control values was noticed even at 4°C after 1½ hours. At 4°C, metabolism is retarded to such an extent that hardly any ATP or substrates will be used or metabolic toxic sub-

![Graph A: Incorporation of 1-14C-leucine into soluble proteins and total lipids respectively of ischaemic rabbit lung slices.](image1)
![Graph B: Incorporation of 1-14C-palmitate into soluble proteins and total lipids respectively of ischaemic rabbit lung slices.](image2)

Fig. 3. The in vitro incorporation of 1-14C-leucine (A) and 1-14C-palmitate (B) into soluble proteins and total lipids respectively of ischaemic rabbit lung slices. The lung tissue was stored at 4° and 37°C for up to 3 hours before the rates of uptake was determined in a Krebs-Ringer bicarbonate medium, pH 7.4, with 10 mM glucose as substrate and in 95% O₂ - 5% CO₂ gas phase. The rates of incorporation of the radiolabelled substrates are expressed as a percentage of the control values. (Each value represents the mean of 3 triplicate determinations.)
stances generated. One would thus expect hardly any irreversible damage to occur at this temperature. However, protein synthesis in ischaemic tissue stored at 37°C for 3 hours is inhibited by 86%. This is certainly the most dramatic effect induced by ischaemia on lung tissue.

The decreased incorporation of 1- 14C-leucine into lung protein is accompanied by a decrease in the amount of soluble, extractable protein from the ischaemic tissue, kept at 4°C and 37°C as shown in Table I. The decrease in protein content could be due to the activation of lysosomal proteases by the ischaemic conditions, with ensuing release of some intracellular proteins into the medium, or to a lesser extent to structural changes in intracellular soluble proteins due to an accumulation of calcium during ischaemia.5

It has been shown that ischaemia lasting 3 hours causes irreversible liver cell injury accompanied by loss of almost one-half of total phospholipids.9 Considering the protein loss which occurred in lung tissue incubated for 2 hours and stored at 4°C and 37°C after 1 1/2 hours, the reduction in the amount of extractable soluble protein from the ischaemic tissue at 37°C was significantly greater than that from ischaemic tissue at 4°C. This could be due to the fact that lysosomal proteases would hardly hydrolyse proteins at 4°C. Structural changes due to calcium accumulation could be expected to be very similar under our experimental conditions, and could therefore not account for the protein loss during the incubation period. Recovery experiments (not reported here) showed that almost all of the protein could be found in the incubation medium. Therefore, membrane disintegration must have occurred, resulting in the solution of cytosol proteins.

Judged by the rate of 1- 14C-palmitate incorporation into the lipids of ischaemic lung tissue stored at 4°C and 37°C, a significant reduction had taken place, very similar to the retarded 1- 14C-leucine incorporation into proteins. The rate of incorporation is inversely related to the temperature during ischaemia and is thus much slower when tissue is stored at 37°C than at 4°C. Whether this decreased rate of incorporation was due to a loss of total phospholipids from the ischaemic lung could not be ascertained from our results. Farber et al.10 found accelerated phospholipid degradation in ischaemic liver cells, resulting from the activation of membrane-bound phospholipases. It is known that lung tissue is rich in phospholipids, but whether microsomal membrane phospholipases of lung tissue are activated by ischaemia was not determined. From indirect calculations of total lipids in normal and ischaemic lungs as used in our experiments, it would appear that the total lipid content is not affected by severe ischaemia. However, at 37°C the rate of 1- 14C-palmitate incorporation into the phospholipid complex of ischaemic lungs is markedly reduced. Seeing that the type II alveolar cells are involved in phosphatidyl choline synthesis, it would appear that these cells must be particularly sensitive to ischaemic damage or that they possess a phospholipase which is activated by ischaemia. Although one could not judge the loss in total phospholipid content from our findings, the rate of 1- 14C-palmitate incorporation into total phospholipids and especially into the dipalmitoyl phosphatidyl choline fraction of ischaemic lung tissue stored at 37°C was significantly reduced.

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REFERENCES