It is well recognized that inhalation anaesthetic agents exert metabolic effects on many important organs such as the liver and brain. For example, halothane acts on glycogenolytic (Biebuyck and Lund, 1974), mitochondrial (Cohen, 1973) and microsomal (Hallen and Johansson, 1975) pathways within the liver. Although inhalation anaesthetics are introduced through, and so reach their highest concentrations in, the lungs, there is little information available as to their effects on lung metabolism. The majority of previous work has concentrated on the effects of halothane on the uptake and metabolism of vasoactive amines from the pulmonary circulation (Naito and Gillis, 1973; Bakhle and Block, 1976; Watkins, Wartell and Rannels, 1983). However, more recently it was shown that halothane reversibly inhibits protein synthesis in rat lungs (Wartell et al., 1981; Rannels, Christopherson and Watkins, 1983).

The object of the present study was to examine the effects of enflurane and halothane on carbohydrate metabolism in isolated, perfused rabbit lungs.

MATERIALS AND METHODS

Surgical preparation

New Zealand white/lop-eared cross-bred rabbits which had been fed ad libitum, were anaesthetized with thiopentone 25 mg kg$^{-1}$ administered through an ear vein. Tracheotomy was performed, a 6-mm diameter flexible, polyethylene cannula was inserted to the trachea and the lungs ventilated with a mixture of 7% carbon dioxide in 93% oxygen. Heparin 1000 u. kg$^{-1}$ was given i.v., the thorax opened, and after the rabbit had been exsanguinated via the right ventricle, the heart and lungs were excised rapidly. A 4-mm diameter flexible, polyethylene cannula was placed in the pulmonary artery from the right ventricle and secured. A similar cannula was passed through the left ventricle and placed in the left atrium. The pulmonary artery cannula was connected to the bubble-trap of the perfusion circuit and that from the left atrium placed in the perfusion reservoir (fig. 1).

The perfusion medium was Krebs–Henseleit high bicarbonate buffer to which was added bovine albumin (Fraction V, Sigma Chemical...
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Company) and glucose to give final concentrations of approximately 5 mg dl\(^{-1}\) and 5 mmol litre\(^{-1}\), respectively. The perfusate was aerated with the 7% carbon dioxide in 93% oxygen mixture for 1 h before use. The circuit was checked meticulously for air bubbles, which were removed if present. The lungs were suspended from a calibrated weight transducer in a Perspex box so that a continuous measurement of changes in lung weight could be obtained. Pulmonary artery and airway pressures were determined using calibrated pressure transducers.

Perfusion was commenced using a non-pulsatile, recirculating system with the perfusate temperature maintained at 37°C. The flow rate was 20 ml min\(^{-1}\), which was verified at the beginning and end of each experiment. Ventilation of the lungs was undertaken using the carbon dioxide in oxygen mixture with an end-expiratory pressure of 2 cm H\(_2\)O. The ventilator was set initially to deliver a tidal volume of 10 ml kg\(^{-1}\) at a frequency of 25 b.p.m. and adjusted to maintain perfusate values in the range pH 7.42–7.51, \(P_{O_2}\) 35.9–46.6 kPa and \(P_{CO_2}\) 4.9–5.6 kPa. The perfusion pressure in the pulmonary artery was adjusted to 15.0 mm Hg at the start of the study.

The preparation was allowed to equilibrate for at least 1 h before the start of each investigation.

**Enflurane study**

Eight rabbit lungs were ventilated with the 7% carbon dioxide in 93% oxygen mixture for 3 h and a further eight lungs were studied with the addition of 2% enflurane from a calibrated vaporizer to the gas mixture. Perfusate samples were collected simultaneously every 15 min from the pulmonary artery and left atrium for the determination of glucose, lactate and albumin concentrations in duplicate (Dommes, Watson and Biggs, 1971; Hall et al., 1980). The pulmonary artery pressure, airway pressure and lung weight were noted at each sample time. The exchange of metabolites across the lungs was calculated from the product of the arteriovenous concentration difference of the metabolite and the flow rate and expressed as \(\mu\)mol min\(^{-1}\).

**Halothane study**

As the results of the enflurane study showed that prolonged ventilation had little effect on metabolite exchange, a shorter period of perfusion was used in this study. Eight lungs were ventilated with the 7% carbon dioxide in 93% oxygen mixture for 1 h and a further eight lungs were studied with the addition of 1% halothane from a calibrated vaporizer to the gas mixture. Samples of perfusate were collected every 15 min to
determine the exchange of glucose and lactate across the lungs as described above.

At the end of the 1-h perfusion the lungs were frozen rapidly in liquid nitrogen, weighed and freeze-dried to constant weight. The concentrations of glycogen, glucose, glycolytic intermediates and high-energy phosphate compounds in the freeze-dried lung tissue were determined by methods described previously (Hall and Lucke, 1983).

The results are expressed as mean values (±SEM). Statistical analysis of the data was undertaken using two-way or one-way analysis of variance, as appropriate.

RESULTS

Enflurane study

During the 3-h perfusion the increases in pulmonary artery pressure, airway pressure and lung weight were similar in the control lungs and in those ventilated with 2% enflurane (2.7±1.0 and 3.7±1.1 mm Hg; 4.8±1.9 and 2.1±0.6 mm Hg; 0.90±0.25 and 0.64±0.15 g, respectively).

Glucose concentrations in the pulmonary artery were maintained between 5.1 and 5.4 mmol litre\(^{-1}\) in the control group and 5.2 and 5.5 mmol litre\(^{-1}\) in the enflurane group (fig. 2). Glucose utilization did not change significantly during the period of perfusion (3 h), nor was there a significant difference between the two groups. The mean glucose uptake throughout the perfusion was 13.6±0.5 \(\mu\)mol min\(^{-1}\) for the control group and 12.5±0.4 \(\mu\)mol min\(^{-1}\) for the enflurane group.

Lactate concentrations in the pulmonary artery varied between 0.70 and 0.90 mmol litre\(^{-1}\) in the control group, but were slightly greater in the enflurane group: from 0.87 to 1.14 mmol litre\(^{-1}\) (fig. 3). Ventilation with 2% enflurane did not alter significantly the rate of lactate production. Mean rates of lactate efflux for the 3-h perfusion were 8.0±0.3 \(\mu\)mol min\(^{-1}\) (control) and 9.7±0.5 \(\mu\)mol min\(^{-1}\) (enflurane).

The pulmonary artery albumin concentration varied between 5.24 and 5.46 g dl\(^{-1}\). No arteriovenous difference in albumin concentration was detected in either group.

Halothane study

The pulmonary artery pressure, airway pressure and lung weight did not increase significantly during the short perfusion period of 1 h in either group and there was no significant difference between the groups.

Pulmonary artery glucose concentrations were lower in this study (3.9–4.5 mmol litre\(^{-1}\)) and were associated with higher rates of glucose utilization during the perfusion period (19.8±3.0 \(\mu\)mol min\(^{-1}\), control group; 22.5±3.8 \(\mu\)mol min\(^{-1}\), halothane group). Lactate efflux was similar in both groups: 8.2±0.5 \(\mu\)mol min\(^{-1}\) (control) and 9.9±1.1 \(\mu\)mol min\(^{-1}\) (halothane).

Ventilation of the lungs with 1% halothane for 1 h had no effect on tissue concentrations of
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glycogen, glucose, glycolytic intermediates and high-energy phosphate compounds (table I).

The pulmonary artery albumin concentration varied between 4.80 and 5.10 g dl⁻¹ and no arteriovenous difference was detected in either group. The dry to wet weight ratio of the lungs was 0.16 ± 0.02 in both groups.

DISCUSSION

The results show that ventilating the lungs with 2% enflurane or 1% halothane had no effect on carbohydrate metabolism as assessed by changes in glucose uptake, lactate efflux and tissue metabolite concentrations. The ineffectiveness of halothane in altering carbohydrate metabolism is in contrast to its ability reversibly to inhibit protein synthesis in the lungs (Wartell et al., 1981; Rannels, Christopherson and Watkins, 1983). In the studies of protein synthesis, however, the lungs were ventilated with concentrations of halothane as high as 4% for 3 h (46% inhibition); 1% halothane resulted in only a 10% inhibition in the rate of protein synthesis (Rannels, Christopherson and Watkins, 1983). Obviously, the usefulness of data obtained during ventilation with such high concentrations of halothane must be questionable.

The assessment of oedema in the preparation is a vital aspect of any study using perfused lungs (Chang, Wright and Effros, 1981). Most investigators have confined their estimation of oedema to histological examination at the end of the period of perfusion, together with the determination of the dry to wet weight ratio. The ratio is then compared with values obtained from unperfused lungs (Alberti et al., 1978; Woods et al., 1980). This method only gives an estimate of total oedema formation throughout the perfusion, and does not indicate its rate of development. The method of assessment of oedema used in the present study—continuous measurement of pulmonary artery pressure, airway pressure and lung weight—is an improvement on the usual procedure. In particular, the measurement of changes in lung weight was of great value and usually preceded detectable changes in pulmonary vascular resistance. The greatest weight gain recorded during the experiment was 1.5 g, which was only 6% of the lung wet weight.

The ability of the isolated lung to utilize exogenous glucose has been described previously and is influenced by many factors including

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control (μmol/g dry wt)</th>
<th>Halothane (μmol/g dry wt)</th>
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<tbody>
<tr>
<td>ATP</td>
<td>2.9 ± 0.9</td>
<td>1.7 ± 0.8</td>
</tr>
<tr>
<td>Phosphocreatine</td>
<td>17.4 ± 5.6</td>
<td>21.5 ± 6.7</td>
</tr>
<tr>
<td>ΔATP + Phosphocreatine</td>
<td>20.4 ± 6.0</td>
<td>23.2 ± 7.1</td>
</tr>
<tr>
<td>ADP</td>
<td>0.66 ± 0.26</td>
<td>0.43 ± 0.34</td>
</tr>
<tr>
<td>AMP</td>
<td>0.20 ± 0.10</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>Creatine</td>
<td>0.36 ± 0.17</td>
<td>0.15 ± 0.04</td>
</tr>
<tr>
<td>Glycogen*</td>
<td>44.7 ± 8.3</td>
<td>39.7 ± 3.1</td>
</tr>
<tr>
<td>Glucose</td>
<td>9.1 ± 1.8</td>
<td>10.8 ± 1.2</td>
</tr>
<tr>
<td>Glucose-6-phosphate</td>
<td>0.79 ± 0.43</td>
<td>0.34 ± 0.06</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>1.4 ± 0.7</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>Lactate</td>
<td>7.1 ± 1.6</td>
<td>7.1 ± 0.9</td>
</tr>
</tbody>
</table>
starvation, the glucose and lactate concentrations of the perfusate, anoxia, ventilation and insulin (Alberti et al., 1978). It has been assumed that the importance of circulating glucose is related to the small amounts of stored glycogen and triglyceride (Datta, Stubbs and Alberti, 1980). The glycogen contents of the lungs found in the halothane study (table I) confirm the small amount of stored glucose. If the rates of glucose utilization found in the halothane study were supported solely from glycogen, then they would provide sufficient glucose for only 10 min. Lactate is quantitatively the major product of glucose metabolism by lung tissue and can account for up to 50% of the glucose utilization (Fisher and Dodia, 1984). The metabolite concentrations obtained in the halothane study (table I) provide supporting evidence for the glycolytic fate of glucose taken up from the perfusate. After corrections are made for conversion from dry to wet weight, the appropriate concentration gradients were present between lung and perfusate for both metabolites. Fisher and Dodia (1984) noted the extreme sensitivity of lactate production by isolated lungs to the circulating lactate concentration with a decrease from 58% to 5% as the lactate concentration of the perfusate increased from 0 to 2 mmol litre⁻¹. At high circulating lactate values (> 5 mmol litre⁻¹) the lungs show an uptake of lactate and so probably have an important role in the clearance of this metabolite during physiological and pathological lactic acidosis (Datta and Alberti, 1979).

It is important to note that the rates of substrate utilization found in the present study occurred in the presence of high oxygen tensions in the perfusate. However, previous work has shown little effect of changes in oxygen tension on lung metabolism, providing that neither hypoxia nor hyperbaric oxygen are used (Bassett and Fisher, 1979; Watkins and Rannels, 1979).

In conclusion, the data suggest that enflurane and halothane at clinically relevant concentrations, have no effect on carbohydrate metabolism in isolated, perfused rabbit lungs. Direct determination of the glycolytic rate in the lung using radioactivity labelled glucose should be undertaken to confirm these observations.

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