The Effect of Inhalation of Halogenated Anesthetics on Rat Liver Mitochondrial Function

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Liver mitochondria obtained from rats exposed to either 3 per cent or 5 per cent halothane had significantly higher rates of oxygen uptake than did those of a corresponding control series. This increased respiration occurred both in the presence and in the absence of adenosine diphosphate. No significant change in rates of respiration was found with exposure to 1 per cent halothane. The respiratory control ratio and adenosine-di-phosphate-to-oxygen values were not altered by prior halothane anesthesia at any dose level used. Electron microscope study of the mitochondria of the control and experimental groups revealed no morphologic differences. Apparently, therefore, the inhalation of halothane, as administered under the conditions of this study, does not induce any long-lasting impairment of liver mitochondrial function. (Key words: Anesthetics; Diethyl ether; Fluroxene; Halothane; Methoxyflurane; Liver; Mitochondria; Respiration.)

In a recent study of halothane–mitochondria interaction, Cohen and Marshall exposed isolated rat liver mitochondria to various concentrations of halothane. They found that this anesthetic depressed the rate of oxygen uptake and caused loss of respiratory control. These adverse effects were reversible, however, except when toxic doses were given.

Snodgrass and Piras also reported that isolated mitochondria were adversely affected by halothane; specifically, oxidative phosphorylation was uncoupled, profound swelling was induced, the 2,4-dinitrophenol-stimulated adenosine triphosphatase abolished, and oxidation of pyridine nucleotide-linked respiratory substrates suppressed.

Evaluation of these findings suggests the possibility that trauma associated with the isolation technique renders mitochondria more susceptible to the action of halothane than they would be in the cellular milieu. From the clinical aspect, evaluation of the performance of isolated liver mitochondria obtained from animals immediately following inhalation anesthesia with halothane was desirable. This report presents the findings of such a study.

Methods and Material

Adult male Sprague-Dawley rats weighing approximately 300 g were placed in a specially designed anesthesia chamber and administered a mixture of air and anesthetic agent for 60 minutes. Control of mixture composition and flow rate was maintained with a vaporizer (Ohio Vernitrol). The concentration of anesthetic within the chamber was measured by gas chromatography and was within 0.3 percentage units of the mixture composition set by the Vernitrol control. Immediately following 60 minutes of anesthesia the animals were killed by cervical fracture and the livers extirpated. To 3 g liver was added 9 volumes of cold isolation medium which consisted of 0.18 M KCl, 10 mM ethylene diamine tetraacetate (EDTA), and 10 mM tris-HCl, pH 7.2. The tissue was then homogenized in a Potter-Elvehjem vessel with a Teflon pestle. The homogenate was centrifuged at 900 × g to sediment nuclei and in-

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Table 1. Rates of Oxygen Uptake, Respiratory Control and ADP/O Ratios for Succinate Oxidation by Rat Liver Mitochondria from Halothane-treated and Control Rats

<table>
<thead>
<tr>
<th>Dose</th>
<th>Experimental Series</th>
<th>Control Series</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q_{O_2}</td>
<td>RCR</td>
</tr>
<tr>
<td></td>
<td>Without ADP</td>
<td>With ADP</td>
</tr>
<tr>
<td>1 per cent</td>
<td>20.1†</td>
<td>41.6</td>
</tr>
<tr>
<td>3 per cent</td>
<td>54.7†</td>
<td>146.8†</td>
</tr>
<tr>
<td>5 per cent</td>
<td>40.3†</td>
<td>117.3†</td>
</tr>
</tbody>
</table>

* Q_{O_2} = nanomoles O_2/min/mg protein.
† Means based on five rats.
‡ Significantly different from control group as determined by Student's t test (unpaired data, degrees of freedom = 8; P < 0.01).
§ Rate during utilization of 500 nanomoles of ADP.

tact cells. The resulting supernatant fluid was centrifuged at 9,000 × g in a refrigerated IEC B-20 centrifuge to obtain a pellet rich in mitochondria. This pellet was resuspended in fresh isolation medium and centrifuged at 9,000 × g to obtain a “washed” mitochondrial pellet, then suspended in 3 ml of isolation medium per g starting tissue. Liver mitochondria from anesthetized control rats were obtained concurrently with those from anesthetized animals.

Mitochondrial oxygen uptake was determined by means of a biological oxygen monitor (Yellow Springs Instrument Co.). Respiratory rate, adenosine-diphosphate-to-oxygen ratio (ADP/O) and respiratory control ratio (RCR) were calculated according to the method of Chance and Williams. The assay medium contained 2.4 ml of a solution of 0.25 M sucrose, 5 mM tri-succinate, and 0.7 ml of mitochondrial suspension containing 0.8 to 1.2 mg of protein. The assays were conducted at 37°C. Protein was determined by the method of Miller.

Portions of liver from some halothane-anesthetized and some control rats were excised and fixed in cacodylate-buffered glutaraldehyde solution (2 per cent) and subsequently were stained and embedded in 2 per cent osmium tetroxide and Epon 812, respectively.

Results and Conclusions

Liver mitochondria from rats exposed to either 3 or 5 per cent halothane had significa-

Table 2. Effects of Anesthesia with Methoxyflurane (3 Per Cent), Fluroxene (3 Per Cent), and Diethyl Ether (10 Per Cent) on Succinate Respiration of Isolated Rat Liver Mitochondria

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Number of Rats</th>
<th>Q_{O_2}</th>
<th>RCR</th>
<th>ADP/O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without ADP</td>
<td>With ADP</td>
<td></td>
</tr>
<tr>
<td>Methoxyflurane</td>
<td>Experimental 6</td>
<td>22.5†</td>
<td>48.9</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Control 6</td>
<td>20.7</td>
<td>42.4</td>
<td>2.0</td>
</tr>
<tr>
<td>Fluroxene</td>
<td>Experimental 5</td>
<td>44.5‡</td>
<td>121.5‡</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>Control 5</td>
<td>24.3</td>
<td>50.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>Experimental 5</td>
<td>20.7</td>
<td>47.4</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Control 4</td>
<td>20.6</td>
<td>45.1</td>
<td>2.2</td>
</tr>
</tbody>
</table>

* Q_{O_2} = nanomoles O_2/min/mg protein.
† Means based on number of rats indicated.
‡ Significantly different from control group as determined by Student's t test (unpaired data, degrees of freedom = 8; P < 0.01).
§ Rate during utilization of 500 nanomoles of ADP.
cantly higher rates of oxygen uptake than did those from the corresponding control series (table 1). These higher rates of respiration were observed both in the presence and in the absence of ADP. No significant change in rate of respiration was found when 1 per cent halothane was used.

Although the mean rate of respiration in the presence of ADP appears substantially higher with 3 per cent as compared with 5 per cent halothane, the mean difference is not significant when evaluated by Student's t test \( P > 0.10 \).

The RCR and ADP/O values were not altered by prior halothane anesthesia at any dose level used. These two ratios are sensitive indicators of the degree of functional integrity of isolated mitochondria. In the absence of any discernible differences between the control and experimental means of these two indices, we are led to the conclusion that inhalation of halothane, as administered under the conditions of this study, does not cause an impairment of liver mitochondrial function.

The only striking aftermath of halothane anesthesia in isolated rat liver mitochondria was the twofold increase in rate of respiration either in the presence or in the absence of phosphate acceptor. Cohen and Marshall found above-normal control rates of respiration in the absence of ADP following a 20-minute resuscitation period during which air was passed through the suspension of mitochondria after an initial 20 minutes of exposure to 3.9 per cent halothane. This overshoot in state 4 respiration was interpreted as loss of respiratory control, that is, failure to maintain a low rate of respiration when phosphate acceptor is absent. Resuscitation with air also restored state 3 respiration, although not beyond the rates observed in control mitochondria.

The procedures employed in the present study to isolate mitochondria following halothane anesthesia could have provided a basis for resuscitating mitochondria which were depressed in situ. That is, since the tissue was initially taken up in nine volumes of isolation medium and the mitochondrial pellet was washed once in fresh medium, ample opportunity was provided for removal of all but trace amounts of halothane. With these considerations in mind, there may not be a discrepancy between the findings reported herein and those previously described by Cohen and Marshall and by Snodgrass and Piras using in-vitro models. In any event, the inhalation of halothane, as administered under the conditions of this study, did not induce any long-lasting impairment of liver mitochondrial function.

Electron micrographs revealed no detectable differences between the ultrastructural appearances of mitochondria derived from control animal livers and those from halothane-anesthetized animals.

Table 2 presents data from studies of liver mitochondria from animals that had been subjected to 60 minutes of anesthesia with some other commonly used volatile anesthetics. Tri-fluorocetylvinyl ether (fluoroxene) had the same effect as halothane in increasing state 4 and state 3 respiration rates, but did not significantly alter either the ADP/O or RCR values. Methoxyflurane, another halogenated anesthetic, did not give rise to the increased rates of respiration observed with halothane and fluoroxene, nor did diethyl ether. Of interest in this connection is Van Dyke's report that methoxyflurane and diethyl ether have a much lower propensity for binding to components of biological membranes than does halothane. Perhaps the twofold increase in mitochondrial respiratory rates observed after halothane anesthesia is related to its ability to bind avidly to membranes.

References


