Depression of Oxygen Uptake in Cell Culture by Volatile, Barbiturate and Local Anesthetics

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The rate of oxygen consumption of a suspension of mouse heteroploid cells was depressed by volatile, local and barbiturate anesthetics. Concentrations of volatile and local anesthetics that halved the oxygen consumption were quite similar to concentrations associated with clinical anesthesia. However, barbiturates were relatively ten times less potent in vitro. The reduction in oxidative metabolism appeared to be regularly associated with increased glycolysis.

Potency and ratio of effective dose to overdose are important clinical characteristics of anesthetics and constitute fundamental data in the study of mechanisms of action. Comparisons of these properties in different classes of anesthetic drugs has been hampered by the lack of tests equally applicable to volatile, barbiturate and local anesthetics. The common pharmacologic criterion of potency, the dose effective in abolishing a specific response in half the subjects (ED₅₀), can be assessed in the case of the volatile anesthetics by measuring the alveolar partial pressure of vapor after equilibrium with key tissues is approached. With nonvolatile agents such as the barbiturates and local anesthetics, however, it is difficult to produce a steady blood level in the blood or tissues, and the effective concentrations are not accurately known. The ED₅₀ itself does not define the margin of safety and, for this purpose, must be supplemented by measurement of the median overdose, such as the dose producing ventilatory or circulatory arrest. Such measurements tend to lack precision because anesthetic depression of the nervous system may be reinforced by secondary blood gas changes that obscure the part played by the anesthetic.

At the price of artificiality, we have avoided the above-mentioned uncertainties by working with a controllable in-vitro system which provides quantitative comparisons of the ability of anesthetics to depress oxidative metabolism. The relevance of such a study arises out of the fact that metabolic alterations, although rarely credited as the cause of general anesthesia, do accompany it and in all probability underlie some of the toxic complications. Depression of cerebral oxygen consumption is known to occur in the presence of halothane and thiopental, both during anesthesia, and in vitro. It seemed worthwhile, therefore, to test the effects of a broad range of anesthetics on oxygen consumption in a standardized in vitro model. The model we have used is a suspension of mouse heteroploid cells from monolayer cultures. We selected this particular system because of its rapid growth, low rate of glycolysis, and lack of tumorigenicity.

Methods

The methods of preparing the cell suspension and measuring oxygen uptake polarimetrically have been described. In brief, cells cultured in monolayer in 32-ounce prescription bottles for four days were detached by trypic digestion, washed and suspended in fresh culture medium. The cell population of an aliquot was determined with an electronic counter (Coulter) and measurements of oxygen consumption of the remainder were made. The suspensions, constituted at random, contained from 2.5 to 10 × 10⁶ cells per ml. A tissue respirometer (capacity 0.93

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ml) at 37 C was filled with the suspension, and control measurements of the rate of oxygen uptake were made. Following this, a saturated solution of anesthetic in culture medium at room temperature was added to the respirometer in successive measured amounts from a micrometer syringe. The final partial pressures of anesthetic were calculated from the dilution factor, as previously described. The rate of oxygen uptake decreased at once after each addition of anesthetic, reaching a new steady rate within two minutes. The rates during the following five minutes form the basis of this report. Typically, a day's work included measurements of six samples of the same suspension, spread over a period of six hours; the same anesthetic was used in all six samples, each of which was subjected to control measurements and to tests with four or five concentrations of the anesthetic.

Results

The results are summarized in the dose-response curves of figures 1, 2 and 3, relating to inhalation anesthetics, barbiturates and local anesthetics, respectively. For the purpose of quantitative comparisons, we arbitrarily use the "ID₅₀" defined as the interpolated concentration measured on the dose-response curve, that produced 50 per cent inhibition of oxygen uptake. Within each category of anesthetics, the order of potency of the drugs as depressants of oxygen uptake of mouse heteroploid cells generally agreed with their order of potency as clinical anesthetics. The agreement was closest in the case of the volatile anesthetics (fig. 1).

The approximately parallel slopes of the curves for methoxyflurane, halothane, chloroform and diethyl ether indicate that the drugs probably act on oxygen uptake by similar, or at least equally responsive, mechanisms. The fluroxene dose-response curve appears somewhat flatter, suggesting less sensitivity and,

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FIG. 1. Effects of volatile anesthetics on rate of oxygen uptake by a suspension of mouse heteroploid cells. Control rate = 100 per cent. Anesthetic concentrations are expressed as partial pressures in equilibrium with a gas phase where a partial pressure of 0.01 atm is equivalent to approximately 0.4 mM, since an ideal gas at standard pressure contains 1 mole per 25 liters, or 40 mM/L when the temperature is 30.4 C. The number of experiments with each drug is given in parentheses. Bars show ± standard error.

§Agla Syringe, Burroughs Wellcome and Co., 1 Searsdale Road, Tuckahoe, New York.

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FIG. 2. Effects of barbiturates. Stimulation of oxygen uptake by low concentrations of thiobarbiturates suggests uncoupling of oxidative phosphorylation.

FIG. 3. Effects of local anesthetics on rate of oxygen uptake by the cell system. There is exceptional sensitivity to dibucaine.
Table 1. Potencies of Volatile Anesthetics as Inhibitors of Mouse Heteroploid Cell Respiration and as Anesthetics in the Dog

<table>
<thead>
<tr>
<th></th>
<th>Cells: ID&lt;sub&gt;50&lt;/sub&gt; (atm x 10&lt;sup&gt;-7&lt;/sup&gt;)</th>
<th>Dog: Anesthetic Pressure (atm x 10&lt;sup&gt;-7&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methoxyflurane</td>
<td>0.24</td>
<td>0.23</td>
</tr>
<tr>
<td>Halothane</td>
<td>0.77</td>
<td>0.87</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>6.4</td>
<td>3.04</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>7.6</td>
<td>5.99</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.93</td>
<td>—</td>
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Possibly, a somewhat different mechanism of action.

With the barbiturates (fig. 2), the concentrations required to depress oxygen uptake by the cell culture by 50 per cent are at least ten times higher than those prevailing in the blood stream during drug-induced sleep. The high lipid:blood partition coefficient of barbiturates may partly account for this. The difference would also appear smaller if we had selected a lesser degree of in vitro inhibition as the basis for comparison. Nevertheless, taking the ID<sub>50</sub> as the criterion, it is clear that the order of potency with respect to inhibition of oxygen uptake in vitro does resemble the order of hypnotic potency in vivo. The oxybarbiturate curves fall into two groups, amobarbital, pentobarbital, methohexitol and secobarbital, all with ID<sub>50</sub> values approaching 1 mM/l, and phenobarbital with an ID<sub>50</sub> many times greater.

The thiobarbiturates, thiopental and thiophenyl, in sufficiently low concentrations, actually accelerate the oxygen uptake, presumably an expression of the uncoupling of oxidation observed by Aldridge and Parker with thiobarbiturates in liver mitochondria. No such stimulation could be demonstrated with any of the other drugs mentioned in this report.

Among the local anesthetics (fig. 3), the response curves of procaine, propoxycaine (Procaine) and lidocaine (Xylocaine) are clustered on the right, showing that these drugs are roughly equipotent in the cell system, with ID<sub>50</sub> values of about 12 mM (0.283 g per 100 ml in the case of procaine). Tetraclane (Pentacaine), with an ID<sub>50</sub> of 0.8 mM, is 15 times more potent than procaine, but the slope of the tetraclane curve is roughly parallel to that of the first three, implying substanc-

Table 2. Effects of Volatile Anesthetics on Multiplication and Carbohydrate Metabolism of Mouse Heteroploid Cells in Monolayer Cultures

<table>
<thead>
<tr>
<th>Vapor</th>
<th>Vapor Pressure (atm x 0.01)</th>
<th>Day 1&lt;sup&gt;st&lt;/sup&gt; Population (cells x 10&lt;sup&gt;9&lt;/sup&gt;)</th>
<th>Glucose Utilized (µM/cell x 10&lt;sup&gt;9&lt;/sup&gt;)</th>
<th>Lactate Produced (µM/cell x 10&lt;sup&gt;9&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.94</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.80</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.53</td>
<td>31</td>
<td>62</td>
</tr>
<tr>
<td>Fluroxene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.30</td>
<td>35</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0.27</td>
<td>49</td>
<td>110</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.90</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.50</td>
<td>16</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.10</td>
<td>70</td>
<td>135</td>
</tr>
<tr>
<td>Halothane</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0.93</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.71</td>
<td>18</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>0.45</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Methoxyflurane</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>0.77</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.61</td>
<td>22</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.40</td>
<td>27</td>
<td>72</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.918</td>
<td>5</td>
<td></td>
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</tbody>
</table>

* The results with each anesthetic are the means from four replicate cultures in a single experiment.
** Day 0 inoculum: 98,000 cells.
Depression of oxygen uptake in cell culture...
oxidative metabolism of neural tissue in vitro respond to anesthetics to similar degrees. The cells in our control preparations appeared to maintain uninhibited, maximal metabolism. Thus, the susceptibility of the oxidative metabolism of these cells to local and volatile anesthetics suggests that in an animal organism tissue cells operating near the limit of their oxidative capacity are also the ones most likely to show metabolic and functional impairment in the presence of these anesthetics. They's observation in that the dog 0.8 per cent halothane vapor produces a 17 per cent decrease in myocardial oxygen uptake is of interest in this connection.

Summary

Volatile, barbiturate and local anesthetics were compared with respect to their ability to depress oxygen uptake of a suspension of mouse heteroploid cells. The partial pressures or concentrations producing 50 per cent inhibition (ID_{50}) were:

- methoxyflurane 0.0024 atm
- halothane 0.0077
- chloroform 0.0093
- diethyl ether 0.064
- fluoroxene 0.076
- secobarbital 0.7 mM/l
- pentobarbital 1.0
- methohexital 1.1
- amobarbital 1.6
- thiopental 3.5
- phenobarbital 19
- tetracaine 0.81
- dibucaine 1.5
- lidocaine 12
- procaine 12
- propitocaine 13

The ID_{50} concentrations were similar in magnitude to the concentrations associated with clinical anesthesia, except in the case of the barbiturates, which were about ten times less potent in vitro. The order in each group paralleled the order of potency in producing depression of the mammalian nervous system. At least in the case of volatile anesthetics, the inhibition of oxygen uptake was not an isolated phenomenon, but was always accompanied by a corresponding increase in glycolysis.

References


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