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

B. Raymond Fink, M.D.,* George E. Kenny, Ph.D.,† William E. Simpson, III

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 have 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 (ED$_{50}$), 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.$^{1,2}$ 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,$^{3}$ and the effective concentrations are not accurately known. The ED$_{50}$ 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,$^{4,5,6}$ and in vitro.$^{7,8}$ 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.$^{9}$ 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 $10 \times 10^6$ cells per ml. A tissue respirometer $\dagger$ (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 "ID_50", 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 fluoxetine dose-response curve appears somewhat flatter, suggesting less sensitivity and,

**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.
### 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: ID₅₀ (atm × 10⁻⁷)</th>
<th>Dogs¹: Anesthetic Pressure (atm × 10⁻²)</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>Fluroxene</td>
<td>7.6</td>
<td>5.99</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.93</td>
<td>—</td>
</tr>
</tbody>
</table>

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.¹⁰ 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 ID₅₀ 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, methohexital and secobarbital, all with ID₅₀ values approaching 1 mM/l, and phenobarbital with an ID₅₀ many times greater.

The thiobarbiturates, thiopental and thiamylal, 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 ID₅₀ values of about 12 mM (0.23 g per 100 ml in the case of procaine). Tetracaine (Pontocaine), with an ID₅₀ of 0.8 mM, is 15 times more potent than procaine, but the slope of the tetracaine curve is roughly parallel to that of the first three, implying substan-

### 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 × 0.01)</th>
<th>Day 14° Population (cells × 10⁹)</th>
<th>Glucose Utilized (µM/cell × 10⁹)</th>
<th>Lactate Produced (µM/cell × 10⁹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>0.5</td>
<td>0.994</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.898</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.538</td>
<td>31</td>
<td>62</td>
</tr>
<tr>
<td>Fluroxene</td>
<td>8</td>
<td>0.308</td>
<td>35</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0.271</td>
<td>49</td>
<td>110</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>3</td>
<td>0.908</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.506</td>
<td>16</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.100</td>
<td>70</td>
<td>135</td>
</tr>
<tr>
<td>Halothane</td>
<td>0.4</td>
<td>0.933</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.711</td>
<td>18</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>0.458</td>
<td>33</td>
<td>37</td>
</tr>
<tr>
<td>Methoxyflurane</td>
<td>0.125</td>
<td>0.778</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.619</td>
<td>22</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.640</td>
<td>27</td>
<td>72</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.918</td>
<td>5</td>
<td>5</td>
</tr>
</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

Discussion

The partial pressures of volatile anesthetics producing 50 per cent depression of oxygen uptake in mouse heteroploid cells happen to be almost identical to the anesthetizing doses of volatile anesthetics in the dog, as shown in table 1. This table compares the ID$_{50}$ values for methoxyflurane, halothane, diethyl ether, fluroxene and chloroform with the so-called minimum anesthetic concentrations determined by Eger and associates. The correlation could be fortuitous and does not necessarily imply that these anesthetics produce their anesthetizing effects on the nervous system through a disturbance of oxidative metabolism. Nevertheless, the observations do support the idea that, when impaired oxygen uptake by the brain occurs during anesthesia, part of the impairment may be due to direct depression of the cellular metabolism.

In a previous study principally concerned with halothane, we showed that depression of oxygen uptake by the heteroploid cell system was accompanied by increased glycolysis. The shift to nonoxidative carbohydrate metabolism was pronounced even with moderate clinical concentrations of this anesthetic, and within limits was a logarithmic function of the partial pressure of the vapor. Using the same methods as before, we now report (fig. 1) that all the volatile anesthetics examined produce analogous effects on oxygen uptake in the cell system and cause corresponding increases in glycolysis (table 2). Many nonvolatile anesthetics are known to produce this effect in vitro in various mammalian tissues, including the brain, kidney, and testis; we have also demonstrated this property for amobarbital in the mouse heteroploid cell system. We generalize these observations to mean that, at constant temperature, diminution of oxygen uptake during in vitro narcosis manifests not so much a decrease in cellular metabolism as a shift to nonoxidative metabolism, signaling an increased and energetically less efficient turnover of substrate.

As regards the results with barbiturates, comparisons with earlier results of studies of metabolism of excised brain tissue in the Warburg apparatus (table 3) indicate that the oxidative metabolism of the mouse heteroploid cells is affected by these drugs to much the same extent as that of suspensions of whole brain.

In order to evaluate the effects of the local anesthetics, we juxtapose some of our results with the responses of slices of rat brain cortex reported by Geddes and Quastel. In table 4, the first two columns show the inhibition of respiration observed by these authors. The last column gives the interpolated values on our own curves at the anesthetic concentrations used by Geddes and Quastel. It will be seen that the sensitivity of the respiration of the mouse-cell system to local anesthetics is just as great as that of the brain slices. An idea of the efficacy of the ID$_{50}$ concentration of procaine (12 mM) in blocking the propagation of nerve impulses is suggested by results obtained by Truant and Takman with squid nerve fibers: immersion of the fibers in a 10 mM solution of procaine interrupted conduction in 9.8 minutes. We also note that the sensitivity of the oxygen uptake by the heteroploid cell to inhibition by anesthetics is of the same order as the sensitivity of the incremental oxygen uptake by stimulated brain tissue and stimulated frog nerve in vitro.

We conclude that wherever data are available for comparison, the oxidative metabolism of the mouse heteroploid cell system and the

| Table 3. Inhibition of Respiration by Barbiturates in Suspensions of Rat Brain and Cultured Cells |
|---------------------------------|-----------------|
|                                  | Rat Brain* (ID$_{50}$, mM) | Mouse Heteroploid Cells (ID$_{50}$, mM) |
| Pentobarbital                   | 1                | 1               |
| Amobarbital                     | 1                | 1.6             |
| Phynobarbital                   | 15               | 19              |

Table 4. Inhibition of Respiration by Local Anesthetics in Slices of Rat Brain and Suspensions of Cultured Cells

<table>
<thead>
<tr>
<th></th>
<th>Rat Brain Slices</th>
<th>Mouse Heteroploid Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procaine, 10 mM/l (2.36 mg/ml)</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>Lidocaine, 5 mM/l</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>Tetracaine, 1 mM/l</td>
<td>19</td>
<td>55</td>
</tr>
<tr>
<td>Dibucaine, 0.5 mM/l</td>
<td>19</td>
<td>13</td>
</tr>
</tbody>
</table>

* See ref. 16.

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 that in 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: atm. 0.0024
- halothane: atm. 0.0077
- chloroform: atm. 0.0093
- diethyl ether: atm. 0.064
- fluroxene: atm. 0.076
- secobarbital: atm. 0.7
- pentobarbital: atm. 1.0
- methohexital: atm. 1.1
- amobarbital: atm. 1.6
- thiopental: atm. 3.5
- phenobarbital: atm. 19
- tetracaine: atm. 0.81
- dibucaine: atm. 1.5
- lidocaine: atm. 12
- procaine: atm. 12
- propitocaine: atm. 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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