Additive Effect of Nitrous Oxide and Halothane on Mitochondrial Function

Michael L. Nahrwold, M.D.,* and Peter J. Cohen, M.D.†

Using lipid solubility as an index of in-citro potency, the concentration of N₂O or halothane necessary for 17 per cent inhibition of state 3 glutamate oxidation by rat liver mitochondria (ID₁₇) was calculated. Exposure to ½ ID₁₇ of N₂O or halothane alone did not significantly alter mitochondrial respiration. However, a mixture of ½ ID₁₇ N₂O plus ½ ID₁₇ halothane depressed oxygen uptake to the same degree as 1 ID₁₇ of halothane. Thus, the depressant effect of this N₂O-halothane mixture on mitochondrial respiration is additive.

(Key words: Nitrous oxide; Halothane; Mitochondria; Respiration; Theories of anesthesia.)

Previous studies in this laboratory have shown that halothane, methoxyflurane, diethyl ether, fluoxetine, enflurane, and isoflurane produce dose-related inhibition of state 3 glutamate oxidation in isolated rat liver mitochondria. The concentration of anesthetic necessary for 50 per cent inhibition of oxygen uptake (ID₅₀) is related to lipid solubility (O/G) such that

$$\log \text{ID}_{50} = 2.746 - 1.035 \log \text{O/G}. $$

In preliminary experiments, 65 per cent N₂O was found to have no effect on mitochondrial respiration. This is not surprising since solution of the above equation for N₂O (O/G = 1.4) predicts an ID₅₀ of 393 per cent.

Eger’s group has studied combinations of halothane with ethylene or xenon to determine the minimum alveolar concentration of anesthetic (MAC) required to prevent gross movement in response to surgical incision in 50 per cent of patients. They found that the effects of anesthetic combinations are additive, i.e., ½ MAC of halothane plus ½ MAC of ethylene or xenon is equal in potency to 1 MAC of any of the three anesthetics studied. Since potency in vivo (MAC) and potency in vitro (ID₅₀) both correlate well with lipid solubility, perhaps ½ ID₅₀ of N₂O mixed with ½ ID₅₀ of halothane are equal in potency to 1 ID₅₀ of halothane. Since the predicted ID₅₀ for N₂O is 393 per cent, this hypothesis cannot be tested unless facilities for delivering N₂O at pressures greater than one atmosphere are at hand.

This difficulty can be overcome by using a concentration lower than the ID₅₀. Since previous experiments in this laboratory have utilized anesthetics vaporized in air, we elected to replace the nitrogen in air with N₂O. In Denver (elevation one mile above sea level, average ambient atmospheric pressure 630 torr), Pₙ₂ₐ = 498 torr. This is equivalent to (498/760)-100 = 65 per cent nitrogen at sea level. Thus, N₂O equivalent to 65 per cent of a standard atmosphere would replace the nitrogen in air in Denver.

What is the predicted inhibition following exposure to 65 per cent N₂O? Inhibition of mitochondrial respiration varies linearly with anesthetic concentration provided that inhibition is ≤ 50 per cent. Thus, exposure to 65 per cent N₂O would be expected to produce (65/393) 50 per cent = 8.3 per cent inhibition. This is approximately equivalent to ½ the concentration necessary for 17 per cent inhibition, i.e., ½ ID₁₇. Since the predicted ID₅₀ for halothane (O/G = 224) is 2.06 per cent, the ID₁₇ for halothane can be predicted as (17/50)-2.06 per cent = 0.70 per cent. Using

* Instructor.
† Professor and Chairman.

Received from the University of Colorado Medical School, Department of Anesthesiology, 4200 East Ninth Avenue, Denver, Colorado 80220. Accepted for publication January 30, 1973. Supported in part by Veterans Administration Grant 1500-01.

1 Except as otherwise noted, all anesthetic concentrations are expressed as percentages of a standard atmosphere.
TABLE 1. Effects of N2O, Halothane, and a N2O–Halothane Mixture on State 3 Glutamate Oxidation by Rat Liver Mitochondria

<table>
<thead>
<tr>
<th>N2O Concentration (Per Cent)*</th>
<th>Halothane Concentration (Per Cent)*</th>
<th>State 3 Oxygen Uptake (nmol/min/mg Mitochondrial Protein)*</th>
<th>Per Cent Inhibition of State 3 Oxygen Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>None (control)</td>
<td>35.44 ± 2.86</td>
<td>—</td>
</tr>
<tr>
<td>—</td>
<td>0.35 ± 0.01</td>
<td>36.89 ± 2.33†</td>
<td>None</td>
</tr>
<tr>
<td>—</td>
<td>0.69 ± 0.01</td>
<td>28.11 ± 2.06†</td>
<td>21</td>
</tr>
<tr>
<td>66.51 ± 0.11</td>
<td>0.35 ± 0.01</td>
<td>33.56 ± 1.89†</td>
<td>5</td>
</tr>
<tr>
<td>66.51 ± 0.11</td>
<td>0.37 ± 0.02</td>
<td>28.89 ± 2.22†</td>
<td>18</td>
</tr>
</tbody>
</table>

* Mean ± SE.
† No difference from control values, P > 0.10.
‡ Different from control values, P < 0.01. No different from each other, P > 0.10. Not different from 29.42 (which would result from 17 per cent inhibition of the control).

these predicted values for ID\(_{50}\), the following study was performed.

Methods

Preparation of rat liver mitochondria, exposure to anesthetic vapor, and polarographic estimation of state 3 oxygen uptake at 25 °C with glutamate as substrate were performed as previously described. Nine experiments were performed, each consisting of an attempt to equilibrate mitochondrial suspensions simultaneously with each of the following gas mixtures: 1) air (control); 2) 0.35 per cent halothane in air (expected to be ½ ID\(_{50}\)); 3) 0.70 per cent halothane in air (expected to be 1 ID\(_{50}\)); 4) 65 per cent N2O (expected to be ½ ID\(_{50}\)); 5) 0.35 per cent halothane–65 per cent N2O (expected to be 1 ID\(_{50}\)).

Halothane was vaporized in temperature- and flow-compensated vaporizers, with concentrations being determined by gas chromatography. N2O concentrations were determined indirectly by measuring oxygen concentration with a Beckman-Pauling Model D2 paramagnetic oxygen analyzer. The analyzer was calibrated with room air and could be read with an accuracy of ±1 per cent oxygen.

The order in which mitochondrial suspensions were analyzed for oxygen uptake was determined by a list of pseudo-random numbers generated by a Hewlett-Packard 9810A desk calculator. Differences between individual groups of data could thus be examined by Student's t test for paired data.

Results

Exposure to 67 per cent N2O or 0.35 per cent halothane did not cause measurable depression of mitochondrial oxygen uptake (table 1). However, equilibration with 67 per cent N2O–0.37 per cent halothane and with 0.69 per cent halothane depressed state 3 respiration by 18 and 21 per cent, respectively. Both these values significantly differ from control, but neither of them differs from the predicted value for 17 per cent inhibition or from the other.

Discussion

The present study tests two hypotheses for a N2O–halothane mixture: 1) the effect of the mixture on mitochondrial function is additive, and 2) the lipid solubilities and concentrations of the gases in the mixture can be used to predict the magnitude of depression of mitochondrial respiration for a given concentration.

One-half ID\(_{50}\) of N2O or halothane does not significantly inhibit mitochondrial respiration. A likely explanation is that 8.5 per cent inhibition is of such low magnitude that it cannot be detected with methods used in the present study. Another possibility is that this concentration is below a threshold required before an effect is observed. Although experiments designed to examine both hypotheses in detail have not yet been performed, data previously obtained for halothane (see figure 3.9 of reference 1) make the former more attractive. In either event, the 18 per cent inhibition follow-
ing exposure to a mixture of halothane and \( \text{N}_2\text{O} \) agrees with the predicted value of 17 per cent. Thus, \( \text{N}_2\text{O} \) does alter mitochondrial function, but mixture with a subinhibitory dose of another anesthetic is necessary to demonstrate this effect. Perhaps exposure to more than 1 atmosphere of \( \text{N}_2\text{O} \) alone would result in measurable inhibition.

The potencies of mixtures of two anesthetics in vivo are determined by adding the respective potencies of the components of the mixtures.\(^4\) Since there is a striking similarity of potencies in vivo and in vitro in terms of lipid solubility,\(^3\) it is not surprising that the net effect of a \( \text{N}_2\text{O} \)-halothane mixture on mitochondrial respiration is also simply additive.

The present study in no way suggests that depression of mitochondrial function and clinical anesthesia have a cause–effect relationship, or that all anesthetic effects are additive. Indeed, in the intact animal a mixture of cyclopropane with \( \text{N}_2\text{O} \) or ethylene produced antagonism when analgesia was examined.\(^7\) However, the ability to use lipid solubility as an index of potencies in vivo and in vitro may suggest similar molecular mechanisms of action.

Halothane used in this study was supplied through the courtesy of Ayerst Laboratories, Inc., New York, New York.

References


Obstetrics

FETAL HEART RATE. PART II

By direct fetal monitoring, 86 women were found to have fetal bradycardia after paracervical block with 160 to 200 mg of mepivacaine. Fetal bradycardia was defined as heart rate less than 110 beats/min. The mean time of onset of bradycardia was 6 to 7 minutes (range 2–20 min) and mean duration was 8 minutes (range 4–20). Sixteen infants had Apgar scores of 3–6 at 1 minute and five infants had scores of 3–6 at 5 minutes. The remainder had scores of 7–10 at both examinations. Mepivacaine had an inconsistent effect on uterine contractility, and bradycardia was not consistently related to change in uterine activity. An ancillary study by these authors (Part I) reported a 24 per cent incidence of fetal bradycardia after paracervical block with mepivacaine.