Nitrous Oxide Inactivates Methionine Synthetase Activity in Rat Testis

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Methionine synthetase (MS) activity in the brain and liver is decreased following nitrous oxide (N₂O) exposure. Since MS is important for DNA synthesis, this interaction would be expected to have the most serious consequences on actively replicating tissue. The authors therefore measured MS activity in rat testes following 1 h exposure to either 10% or 50% N₂O. Animals exposed to 10% N₂O had a 29% reduction in MS activity, and exposure to 50% N₂O caused a 63% reduction in enzyme activity. Testicular MS activity returned to normal by 24–48 h in the 10% exposure group and by 72 h in the 50% exposure group. This biochemical effect on testicular enzyme activity could be the basis for the reported deleterious effects of N₂O on spermatogenesis. (Key words: Anesthetics, gases: nitrous oxide. Enzymes: methionine synthetase. Testis: spermatogenesis; sperm morphology.)

Nitrous oxide (N₂O) oxidizes vitamin B₁₂, rendering it inactive as a coenzyme in several important biochemical reactions.¹⁻³ For example, even short periods of exposure to clinical concentrations of N₂O result in almost total inactivation of methylcobalamin, the co-factor of the enzyme methionine synthetase (MS).⁴ Methionine synthetase catalyzes the conversion of homocysteine to methionine and is important for normal deoxyribonucleic acid (DNA) production. Although MS activity in the liver has been investigated extensively,⁴ no data are available on the normal activity of MS in the testis or its inhibition by N₂O. Yet, the interaction between N₂O and MS would be expected to have the most serious consequences on an organ such as the testis, which contains actively replicating cells. Therefore, we studied the effects of N₂O on MS activity in the rat testis.

Methods

Seventy-two male Sprague–Dawley rats (Hilltop Lab Animals, Chatsworth, California) weighing 200–250 g were bedded on ground corn cob (Bed-O-Cobs, Anderson's Cob Division, Maumee, Ohio) and housed in polypropylene cages with zinc-coated lids. The animals were fed standard rat chow (Wayne Lab-Blox, Allied Mills, Inc, Chicago, Illinois) and tap water ad libitum. The animals randomly were divided into three groups containing 24 animals each. Group 1 (control) animals were exposed to room air only. Group 2 were exposed to 10% N₂O and Group 3 to 50% N₂O. Exposures were performed at the same time in three identical 1,000 l exposure chambers and were for 1 h. The oxygen tension in each chamber was maintained at 0.21 atm. Medical grade N₂O from tanks was delivered to the chambers through plastic tubing at a total maintenance flow of 10 l/min. Carbon dioxide concentrations were less than 0.1%. Baffles and electrical fans were used to insure uniform concentrations of N₂O within the chamber. The N₂O concentration of each chamber was measured continuously with a Miran 1A-1F infrared gas analyzer and maintained within 5% of the desired concentration.

Immediately after exposure, six animals from each group were killed with carbon dioxide. The remaining rats were returned to their cages. Six animals from each group subsequently were killed at 24, 48, and 72 h following exposure.

The right testis was removed from each animal, weighed, and then homogenized with a Brinkman Polytron homogenizer for 20–30 s at three-quarters speed in 5 ml 0.05 M potassium phosphate buffer (pH 7.4). In addition, approximately 1 g of liver was excised and homogenized separately. MS activity in the testis and liver tissue was assayed according to the method described by Koblin et al.⁵ The tissue homogenates were centrifuged in a Sorvall RC2-B centrifuge at 20,000 g for 80 min at 4° C. The protein content of the supernatant was measured by a modification of the Lowry method.⁶ A 200 µl total volume of methionine synthetase reaction mixture contained 20 µmol of sodium phosphate buffer (0.1 M, pH 7.4), 25 µmol of mercaptoethanol, 50 nmol of adenosine methionine, 10 nmol of vitamin B₁₂, 5 nmol of homocysteine (freshly prepared from the lactone form), 120 nmol of methyltetrahydrofolate (1 µCi of 5-[14C]MTHF), and 40 µl either supernatant or buffer. After incubation for 1 h at 37° C, the reaction was stopped by the addition of 800 µl of cold distilled water. The resultant 1.0 ml of mixture was layered on a Dowex® 1-X8 anion exchange resin (200–400 mesh, chloride form) column, previously equilibrated with 0.1 M sodium phosphate buffer (pH 7.4). The eluate was collected after washing with 3 ml of buffer. An aliquot of eluate was
Table 1. Testicular Weight and Exposure to Nitrous Oxide

<table>
<thead>
<tr>
<th>Group</th>
<th>0</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>1.65 ± 0.16</td>
<td>1.59 ± 0.21</td>
<td>1.64 ± 0.13</td>
<td>1.66 ± 0.16</td>
</tr>
<tr>
<td>2. 10% N₂O</td>
<td>1.55 ± 0.13</td>
<td>1.64 ± 0.18</td>
<td>1.67 ± 0.17</td>
<td>1.72 ± 0.15</td>
</tr>
<tr>
<td>3. 50% N₂O</td>
<td>1.68 ± 0.13</td>
<td>1.65 ± 0.11</td>
<td>1.63 ± 0.10</td>
<td>1.74 ± 0.14</td>
</tr>
</tbody>
</table>

Weight in g ± SD, N = 6.

Mixed with 10 ml of Aquasol® 2 and counted in a Beckman LS30 scintillation counter. MS activity was expressed as nanomoles of methionine produced in 1 h per milligram of protein (nmol · h⁻¹ · mg protein⁻¹).

Analysis of variance and the Student Neuman–Keuls post-hoc test were used for statistical comparisons among the groups; P < 0.05 was considered significant.

Results

The appearance and weight (table 1) of the excised testes were the same for each exposure group. The MS activity in the testes of rats exposed to N₂O decreased in a dose-dependent manner (table 2). Immediately after 1 h activity had fallen to 71% of control value following 10% N₂O (P < 0.05) and to 37% following 50% N₂O (P < 0.01). Recovery of MS activity was complete at 24–48 h following 10% N₂O exposure and at 72 h following 50% N₂O exposure.

Normal MS activity in the liver (range 1.0–1.3 nmol · h⁻¹ · mg protein⁻¹) was about 40–80% above the activity in the testis. Activity in the liver decreased and recovered in a manner similar to that for the testis (table 3). The values found for the liver were essentially the same as those reported previously by other investigators.³

Discussion

Nitrous oxide has been shown to decrease the activity of the vitamin B₁₂-dependent enzyme MS in a variety of tissues. For example, MS activity in the mouse liver is depressed severely following exposure to relatively high concentrations (80%) of N₂O.⁴ Activity returns to control levels only after 2–4 days. Our study confirms these findings. Fifty per cent N₂O for only 1 h caused a marked decrease in MS activity in the liver, which required 3 days for full recovery. As little as 0.01% N₂O (1,000 ppm) exposure for 3 days also can cause a significant inhibition of MS activity in the rat liver.⁶ The assay we used to measure MS activity in the rat liver and testis is a sensitive biochemical index for the metabolic changes associated with N₂O exposure.

Our data demonstrate that N₂O interferes with MS activity in the rat testis similar to its effects in the liver. We found that exposure to 10% N₂O for 1 h produced a significant reduction and 50% N₂O a marked reduction in MS activity in the rat testis. MS activity returned to control levels by 24–48 h in the low- (10%) exposure group and by 72 h in the high- (50%) exposure group.

Other effects of nitrous oxide on the testis previously have been demonstrated. Krioke et al. reported that male rats breathing 20% N₂O for 5 weeks had decreased numbers of testicular sperm and had abnormal giant multinucleated cells in their seminiferous tubules.⁷ The earliest evidence of change occurred in some rats after only 2 days of exposure. After 2 weeks, testicular damage was found in all exposed animals. Normal spermatogenesis returned only when these animals breathed room air for at least 72 h. Another study found dose-dependent increases in chromosomal aberrations in spermatogonial cells of rats exposed for 52 weeks to trace combinations of halothane and N₂O.⁸ The reduction in MS activity found in our study would explain these effects of N₂O on cell division and DNA synthesis.

In contrast to these positive studies of cell numbers and chromosomal aberrations, most studies that have looked at sperm morphology following N₂O exposure have been negative. Although a morphologic assay of mouse sperm following in vivo exposure demonstrated that chloroform, trichloroethylene, and enfluran caused significant increases in abnormal sperm, N₂O had no effect.⁹ In addition, Mazze et al. reported that animals exposed to different concentrations of N₂O (0.5, 5.0, or...
50.0%) for 4 h/day, 5 day/week for 14 weeks had no significant differences in testicular weight, percentage of abnormal sperm, or sperm count, when compared with unexposed control animals. Only one study attempted to assess the effects of anesthetic exposure on human sperm morphology. A comparison of sperm counts and sperm shape from semen of anesthesiologists and control subjects noted no differences related to occupational exposure to low levels of anesthetic gases in the workplace. The biochemical effects of N₂O are more likely to lead to interference with cell division rather than to abnormalities in sperm morphology and so would not be evident in these assays.

Occupational exposure to waste anesthetic gases, particularly N₂O, has been implicated as the cause of higher incidences of infertility, spontaneous abortions, and congenital abnormalities in the children of exposed workers. Although vitamin B₁₂ is present in the testis, its function in normal spermatogenesis is unknown. The effects of N₂O on the biochemical activity of testicular tissue has not been studied previously. Our data show that N₂O inactivates MS activity in the rat testis. Vitamin B₁₂ is important for normal male fertility. Vitamin B₁₂ improves sperm motility and enhances artificial insemination in animals. Conversely, low levels of vitamin B₁₂ in semen often are found in men with infertility problems.

Does the depression of MS activity by N₂O have any reproductive consequences, either for patients exposed to clinical concentrations for short periods of time or for workers chronically exposed to trace levels of the gas? Although our preliminary study found that exposure to as little as 10% N₂O can cause measurable biochemical changes in the rat testis, it would be premature to extrapolate these findings to humans because of the known species differences in the response to N₂O between rats and humans. Whether the changes we observed are important for normal male fertility requires further studies.

### References