Title: HALOTHANE AND ISOFLURANE PREVENT THE TERATOGENIC EFFECTS OF NITROUS OXIDE IN RATS, FOLINIC ACID DOES NOT

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Introduction: N_2O causes adverse reproductive effects (i.e., increased fetal wastage and teratogenicity) when administered to experimental animals. It is generally held that these effects occur as a consequence of the oxidation of cobalt in vitamin B_{12} from the active monovalent form (Cob(I)alamin) to the inactive bivalent form (cob(II)alamin). The latter cannot function as a coenzyme for methionine synthase (MS), the critical enzyme in the transmethylation reaction which converts homocysteine to methionine, and 5-methyl tetrahydrofolate to tetrahydrofolate. As a result, the cascade of metabolic events which under normal circumstances leads to the synthesis of DNA is interfered with, resulting in abnormal DNA production (a known factor in teratogenesis). This biochemically based theory of N_2O teratogenesis is so attractive that other possible teratogenic mechanisms have not been considered. However, there are inconsistencies in the biochemical argument. For example, although, low N_2O dosages administered for a few hours cause marked biochemical effects, high concentrations administered for 24 hours are required for teratogenesis. Because we did not believe the issue of the mechanism(s) of N_2O teratogenesis was resolved, we pursued further investigations in this area.

Methods: Three separate experiments of similar design were performed utilizing a total of 336 timed-pregnant Sprague-Dawley rats. In each experiment there were four groups: 1) a control group exposed to air (n=30-40); 2) a group treated with 50-75% N_2O for 24 h on day 8 of gestation (n=20-30); 3) a group treated with one of three test drugs, (i.e., 5 mg/kg/day of folinic acid [F.A.], using a s.c. implanted osmotic minipump on days 5-11 of gestation; 0.35% isoflurane (ISO) [1/4 MAC] or 0.27% halothane (HAL) [1/4 MAC] for 24 h on day 8 of gestation; n=20-30); and 4) a group treated with N_2O (as in group 2) plus one of the three test drugs, as used in group 3 (n=20-30). In pilot studies, 1/4 MAC was the highest concentration of volatile agent which could be administered in combination with 50% N_2O without causing maternal death. On day 20 of pregnancy, cesarean sections were performed and a total of 3189 offspring were delivered and subsequently examined for external, visceral and skeletal anomalies; reproductive indices also were determined. All examinations were made without knowledge of the treatment group. The percentage of abnormal fetuses in each litter of each treatment group was computed and group means were compared with control group means by ANOVA; Student's *t* test, corrected for multiple comparisons, was used as an *a posteriori* test when differences were found with ANOVA. In another experiment, using a total of 65 rats, hepatic MS activity was measured immediately, 24, 48 and 72 hr after 24 hours of treatment with either 50%N_2O, FA + N_2O or HAL + N_2O.

Results: In all three teratology experiments, data from the control groups were totally consistent with each other so these results were combined (table). For the same reason, data from the N_2O alone groups were pooled. Treatment with N_2O alone resulted in increased incidences of: fetal wastage, major visceral malformations (primarily right sided aortic arch), minor skeletal anomalies and skeletal developmental variants. Treatment with either FA, ISO or HAL alone did not result in any abnormalities, all values being similar to control. Treatment with FA + N_2O was essentially the same as treatment with N_2O alone; the only beneficial effect was a partial reduction in minor skeletal anomalies and developmental variants. To the contrary, both ISO and HAL, when administered with N_2O, protected against the adverse reproductive and teratogenic effects of N_2O. More complete protection was seen with HAL, the data for which were indistinguishable from control values. Hepatic MS activity was equally depressed in all groups at all time periods.

Discussion: These data do not support the theory that N_2O causes adverse reproductive and teratogenic effects because it inactivates methionine synthase, thereby decreasing DNA production. Were this the case, FA should have prevented this effect, as it does the hematologic changes secondary to N_2O. Also, it appears that HAL does not act by preventing the effects of N_2O on MS activity. The fact that, in separate experiments, both ISO and HAL reversed the N_2O effects, suggests that our results are not due to chance and that a common protective mechanism is involved. This mechanism is probably multifactorial. HAL and ISO are sympatholytic and they would attenuate the high adrenergic tone that occurs during N_2O anesthesia. This would help preserve uterine blood flow and possibly would modify the adverse effects of adrenergic stimulation on growth of embryonal/fetal cells. It also is possible that both agents reverse other, as yet unknown, biochemical effects.

Summary of reproductive indices and fetal examinations	Cont.	N_2O	F.A.	F.A. +N_2O	ISO	ISO +N_2O	HAL	HAL +N_2O
No. of rats studied	105	76	28	27	30	30	20	20
No. of rats pregnant	95	63	26	22	27	26	19	19
Pregnancy rate (%)	90	83	93	81	90	87	95	95
Implantations/rat	12.4	12.6	13.2	12.3	12.7	12.0	12.6	12.4
Live fetuses/rat	11.8	8.8*	11.8	7.5*	11.8	10.6	12.0	11.9
Mean fetal weight (g)	4.6	4.4	4.8	4.5	4.4	4.4	4.6	4.4
Early resorptions (%)	6.4	22.8*	8.7	30.5*	7.4	12.4	4.4	3.9
Late resorptions (%)	0.1	8.4*	0.0	13.7*	0.0	1.3	0.0	0.0
Total resorptions (%)	6.5	31.2*	8.7	44.2*	7.4	13.6	4.4	3.9
Female fetuses (%)	47	50	63	47	54	54	51	50
Visceral examinations								
Fetuses examined	564	272	161	80	157	136	116	106
Major malform. (%)	0.2	16.9*	0.0	13.4*	0.0	4.7	0.0	0.0
Minor anomal. (%)	8.6	16.1	6.3	12.7	10.0	10.7	8.9	2.5
Skeletal examinations								
Fetuses examined	563	270	157	85	162	139	112	109
Major malform. (%)	0.1	1.6	0.5	0.0	0.0	0.7	1.8	0.0
Minor anomal. (%)	1.0	12.8*	0.9	6.0	3.1	0.6	2.8	1.1
Develop. var. (%)	22.5	47.5*	24.5	41.8*	23.3	34.5	15.7	34.9

* $p < 0.05$