

Rate of Inactivation of Human and Rodent Hepatic Methionine Synthase by Nitrous Oxide

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The rate of inactivation of hepatic methionine synthase by nitrous oxide has been determined in 22 patients undergoing laparotomy during general anesthesia, including 70% nitrous oxide. Mean half-time of inactivation was 46 min. Metabolic consequences of nitrous oxide are, thus, critically dependent on the duration of anesthesia, and are unlikely to be significant during exposures of less than 40 min. Inactivation of methionine synthase is very much more rapid in the rat exposed to 50% nitrous oxide, with a half-time of 5.4 min. (Key words: Anesthetics, gases: nitrous oxide. Enzymes: methionine synthase. Liver: methionine synthase. Vitamins: B₁₂.)

IN 1978, it was shown that exposure of rats to 50% nitrous oxide in oxygen for 30 min resulted in loss of 72% of hepatic methionine synthase activity,¹ and this was followed by abnormal thymidine synthesis after 60 min exposure.² It was subsequently shown that inactivation also occurred in liver biopsies from seven patients anesthetized with nitrous oxide,³ but the onset of inhibition appeared to be much slower and was incomplete during exposures lasting between 75 and 165 min.

The rate of inhibition of methionine synthase is of great practical importance, since it governs interference with thymidine synthesis resulting from clinical anesthesia with nitrous oxide.⁴ Most research in this field has been undertaken in rodents, but existing data suggest that it is probably not valid to extrapolate the time scale to humans. We have, therefore, determined the time course of the rate of inhibition of hepatic methionine synthase under the circumstances of routine surgery in anesthetized patients breathing 70% nitrous oxide. We have compared the results with parallel observations in rats.

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Materials and Methods

PATIENTS

The study comprised two groups of patients. The first group consisted of seven patients scheduled for partial hepatectomy for neoplasm. In the second group, hepatic biopsies were taken from 20 patients scheduled for laparotomy, and this part of the study was approved by the Hospital Ethical Committee. Informed written consent was obtained from the patients by the surgeon responsible for clinical care. No patient had abnormal liver function tests (raised serum bilirubin, aspartate transaminase, or alanine transaminase). Ages ranged from 27-74 yr, and 11 were male (table 1).

ANAESTHESIA AND CLINICAL MANAGEMENT

Clinical management was the choice of the individual anesthesiologist assigned to each patient, most of whom received an opiate and a parasympatholytic drug as premedication approximately 1 h before surgery. Induction was with thiopental 3-4 mg/kg, and a tracheal tube was inserted after administration of a neuromuscular blocking drug (pancuronium, vecuronium, or atracurium). Twenty-two patients were then ventilated with a mixture of 70% nitrous oxide and 30% oxygen (as gauged by the rotameters), which was used as the carrier gas for any volatile anesthetic (table 1). A non-rebreathing circuit was used with a total fresh gas flow ranging from 6-9 l/min. No nitrous oxide was used in five patients who served as controls, and the volatile anesthetic was vaporized in oxygen.

In the seven patients of group I, we assayed a part of the liver which had been resected but which was deemed by the pathologist (H.K.W.) to be free from tumor. In the 20 patients of group II, a wedge biopsy of liver (approximately 800 mg) was taken at a variable time after commencement of administration of nitrous oxide. The five control biopsies were taken at various times (ranging from 35-240 min) after the commencement of anesthesia (table 1).

EXPOSURE OF RATS

Since this study required some very short exposures, a lucite exposure chamber was fitted with a trap door

TABLE 1. Details of Patients

Age	Sex	Pathology	Anesthetic Agents	Time from Induction to Biopsy (Min)	Methionine synthase Activity ($\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{mg}^{-1}$ protein)
46	M	Hepatic neoplasm	N ₂ O, isoflurane, diamorphine	290	0.05
42	F	Hepatic neoplasm	N ₂ O, diamorphine, droperidol	197	0.25
52	M	Hepatic neoplasm	N ₂ O, enflurane	260	0.11
40	F	Hepatic neoplasm	N ₂ O, halothane, isoflurane	200	0.12
58	M	Hepatic neoplasm	N ₂ O, halothane	180	0.06
42	F	Morbid obesity	N ₂ O, isoflurane	150	0.24
66	M	Colonic neoplasm	N ₂ O, morphine	80	0.13
63	F	Cholelithiasis	N ₂ O, morphine	95	0.16
56	M	Rectal neoplasm	N ₂ O, morphine	40	0.19
53	F	Cholelithiasis	N ₂ O, enflurane	50	0.59
50	M	Cholelithiasis	Halothane	55	0.88
60	F	Cholelithiasis	Enflurane	35	0.94
27	F	Hydatid cyst	N ₂ O, enflurane, fentanyl	35	0.93
63	M	Hepatic neoplasm	Enflurane, morphine	210	0.85
67	M	Gastric neoplasm	Enflurane, morphine	40	1.95
60	M	Hepatic neoplasm	Enflurane, morphine	240	1.19
65	M	Cholelithiasis	N ₂ O, morphine	31	0.99
68	F	Cholelithiasis	N ₂ O, morphine	31	1.40
50	F	Cholelithiasis	N ₂ O, isoflurane	75	0.97
68	F	Cholelithiasis	N ₂ O, isoflurane	63	0.94
74	F	Gastric neoplasm	N ₂ O, enflurane	165	0.18
47	M	Lymphoma	N ₂ O, enflurane	35	0.90
53	F	Cholelithiasis	N ₂ O, enflurane	86	0.25
56	F	Cholelithiasis	N ₂ O, enflurane	48	0.50
42	F	Cholelithiasis	N ₂ O, enflurane	62	0.33
49	F	Cholelithiasis	N ₂ O, enflurane morphine	120	0.16
49	F	Cholelithiasis	N ₂ O, enflurane	78	0.32

through which rats could be introduced into the chamber without appreciable change in the gas composition. The chamber (30 l capacity) was flushed with at least 100 l of a 50/50 mixture of nitrous oxide and oxygen, and flow was then maintained at 7 l/min. Batches of six specific pathogen-free Sprague Dawley male rats (age 4 weeks, 160–185 g) were exposed for 5, 10, 20, 40, or 80 min. Controls were six rats exposed to 50% nitrogen/50% oxygen for 80 min (chamber controls) and six rats breathing room air (cage controls). Humidity in the chamber did not rise above 50% and carbon dioxide concentration above 0.2%. At the specified time, rats were removed from the chamber and immediately given a lethal intraperitoneal dose of pentobarbital (60 mg/kg). As soon as consciousness was lost, the rats were exsanguinated by cardiac puncture, and portions of the liver were then removed.

STORAGE AND ANALYSIS OF SAMPLES

All liver specimens were stored in crushed ice and homogenized with 1 ml of potassium phosphate buffer 0.05 M at pH 7.4 within 30 min of collection. After centrifugation at 4° C, the supernatant was decanted and either frozen at –20° C or assayed immediately. All samples were analysed within 1 week of processing. Methionine synthase activity was determined in duplicate using a method based on the transmethylation of ho-

mocysteine to methionine. 5-[¹⁴C] methyl tetrahydrofolate 100 $\mu\text{mol/l}$ (Amersham International, Amersham, UK) and homocysteine were incubated with the supernatant for 1 h, and the ¹⁴C-labelled methionine formed was separated using a Dowex (Sigma Chemical Co., Poole Dorset, UK) 1 \times 400 (Cl⁻) column prior to scintillation counting.⁵ Activities were determined as nmoles of methionine formed per hour at 37° C per mg of protein, which was determined by Lowry's method.⁶ Using this method, the within batch coefficient of variation is 7%.

STATISTICAL ANALYSIS

The simplest model assumes that nitrous oxide causes methionine synthesis to decline exponentially to a background level, which persists after all the enzyme is inactivated, thus:

$$y = y_0 e^{-kt} + b,$$

where y_0 is the initial value and b is the background value for the rate of methionine synthesis.

Curve fitting was undertaken with a micro-computer, starting with a first order Taylor expansion of the basic equation. The derivation was then essentially as for simple linear regression by the method of least squares.⁷ The variance of y was not constant; to allow for this, a "weighting" procedure was used,⁸ assuming y to have a coefficient of variation of 50%. After weighting, the

residuals were both homoscedastic (Bartlett's test) and Gaussian (Shapiro-Francia W' test⁹).

Results

HUMAN DATA

Of the five patients who did not receive nitrous oxide, the mean methionine synthase activity (MSA) was 1.16 nmol · h⁻¹ · mg⁻¹ protein (SD 0.46), and individual values did not correlate significantly with the duration of anesthesia ($r = -0.27$; $P = 0.66$) (table 1).

For patients receiving nitrous oxide, the variability in MSA is clear from figure 1, which also shows the curve of best fit. Values for the parameters with 95% confidence limits were as follows: y_0 1.18 (0.71–1.65) nmol · h⁻¹ · mg⁻¹; k 0.015 (0.007–0.023) min⁻¹; and b 0.071 (–0.014–0.156) nmol · h⁻¹ · mg⁻¹. Half-time was 46 (30–99) min. (Note that the relationship between the decay constant (k) and half-time is clear from analysis of exponential equations.¹⁰)

RATS

Six cage control rats had a mean MSA of 2.83, and six chamber controls (breathing air) 2.70. These were not significantly different, and were pooled to give a mean control value of 2.77 nmol · h⁻¹ · mg⁻¹ protein (SD 0.33).

The onset of inhibition was much more rapid than in the patients. The earliest samples (5 min) showed a mean activity less than 50% of control (fig. 1). Residual activity was attained after 40 min. Values for the parameters with 95% confidence limits were as follows: y_0 2.23 (1.93–2.53) nmol · hr⁻¹ · mg⁻¹; k 0.128 (0.09–0.168) min⁻¹; and b 0.397 (0.28–0.514) nmol · h⁻¹ · mg⁻¹. Half-time was 5.4 (4.2–7.7) min. The differences between humans and rats are significant at the 1% level for all three parameters. In the rat, b differs significantly from zero, but, in humans, it is borderline.

Discussion

This study has shown a major species difference in the rate of onset, but not the intensity, of inhibition of methionine synthase activity. The time scale of data from the rat cannot be extrapolated to humans, and the duration of anesthesia is a critical factor in the anticipation of metabolic disturbances due to nitrous oxide.

The mean control data for methionine synthase activity of the rats is within the normal range for our laboratory. The interpolated value for 30 min was 17% of control, which agrees with our previous observations^{1,2} and those in mice reported by Koblin *et al.*¹¹ However, there have been no reported observations for exposure periods of less than 30 min, and it was not known that inactivation was extremely rapid with a half-time of only

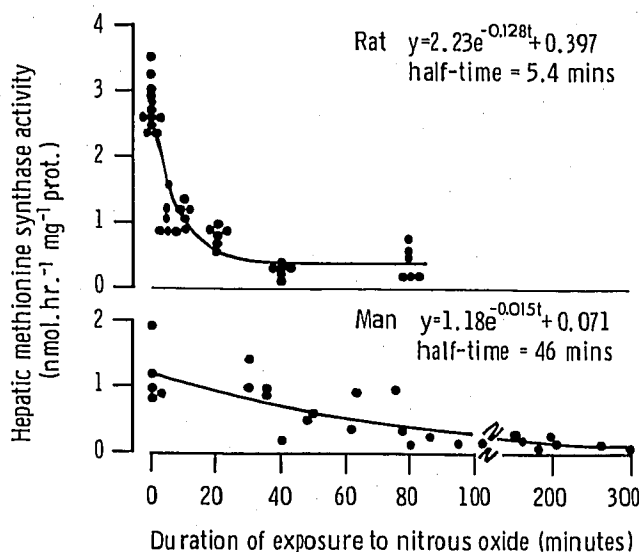


FIG. 1. Time course of inactivation of hepatic methionine synthase activity during exposure to 70% nitrous oxide in humans (lower curve) and 50% nitrous oxide in rats (upper curve).

5.4 min. Background methionine synthase has been observed for as long as 8 days exposure to nitrous oxide.¹²

Taking human liver biopsies for experimental purposes imposes certain constraints. First, we took the minimal number of specimens to reach our conclusions and felt obliged to accept the wide limits of standard error of the mean for controls; this could only have been reduced appreciably by including many more control patients. Second, it was not deemed reasonable to take serial samples which would have enabled us to use patients as their own controls, and would have improved our definition of time course of inhibition and recovery.

Few values for human hepatic methionine synthase activity have been reported. Those of Koblin *et al.*³ cannot be directly compared because they are expressed per gram of liver. However, on the assumption that liver is 10% protein,¹³ Koblin's mean control values would correspond to 4.14 and Baumgartner's adult samples to 2.19 nmol · h⁻¹ · mg⁻¹. Our mean value of 1.16 may, however, be compared with 1.7 nmol · h⁻¹ · mg⁻¹ of protein for normal human placenta.¹⁴ Hepatic enzyme activities are usually less in humans than in rats. The rate of inhibition of MSA is clearly very much slower than in the rat, but not as slow as indicated by the seven biopsies reported by Koblin *et al.*,³ in which a mean concentration of 61% nitrous oxide was used. Kano *et al.*¹⁵ reported very much slower changes in bone marrow with a half-life of the order of 6 h. No changes were found in human placenta follow-

ing maternal exposure to nitrous oxide lasting 13–22 min.¹⁴ Apart from B₁₂ deficiency, no pathological condition is known to alter MSA, and a wide range of volatile anaesthetics have been shown to be without effect.^{2,11} We are unaware of studies of the effect of the other drugs given to these patients.

There is considerable individual variation in the rate of inhibition of methionine synthase activity in specimens taken from patients. Some normal values were found up to 75 min of exposure to nitrous oxide, while one grossly abnormal value appeared as early as 40 min. Thus, there is a wide time range during which prediction of the effect of nitrous oxide is very uncertain. However, our results support the placental data that exposures of less than 25 min are probably harmless.¹⁴

The most serious effects of inhibition of methionine synthase activity relate to interference with deoxyribonucleic acid (DNA) synthesis, which has been detected after as little as 2 h exposure to nitrous oxide.^{16,17} This appears to be consistent with the time course observed in the present study, although the precise quantitative relationship between inhibition of methionine synthase activity and interference with thymidine synthesis has not yet been established for humans. The relationship could be further complicated by other factors influencing levels of methionine and tetrahydrofolate, which might be important in critically ill patients receiving nitrous oxide.¹⁶ Recovery of methionine synthase activity is known to be much slower than inhibition in the rat,² and it is not, therefore, surprising that repeated short exposures may be followed by interference with DNA synthesis.¹⁸

The cause of the major difference in time course between rats and humans is not immediately obvious. It is much greater than could be explained by differences in the time required for equilibration of partial pressure of nitrous oxide between liver and inspired gas which, in humans, is 90% complete within a few minutes.¹⁹ A possible explanation is species differences in the structure of the apoenzyme, with altered access of nitrous oxide to the cobalt of the vitamin B₁₂. However, whatever the explanation, it is evident that any effects following routine anesthesia will depend on the duration of anesthesia. This may well be a factor in the lack of teratogenic effects observed following nitrous oxide anesthesia in early pregnancy,²⁰ since the majority of anaesthetics given during pregnancy are of short duration.

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