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Preoperative Methionine Loading Enhances Restoration of the Cobalamin-dependent Enzyme Methionine Synthase after Nitrous Oxide Anesthesia

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Background: Prolonged exposure to nitrous oxide causes adverse effects mimicking those of cobalamin deficiency. This is explained by irreversible oxidation of cobalamin bound to the enzyme methionine synthase. The inactivation of methionine synthase by nitrous oxide in cultured human fibroblasts is decreased at high concentrations of methionine in culture medium.

Methods: We investigated the possible protection against cobalamin inactivation by preoperative methionine loading in patients undergoing nitrous oxide anesthesia. Fourteen patients receiving anesthesia for 75–230 min were included. Half of these patients received a peroral methionine loading dose 2 h before anesthesia.

Results: After nitrous oxide exposure, a considerable inactivation of methionine synthase in mononuclear white blood cells was seen in all patients, reaching a nadir after 5–48 h. In the patients not subjected to a methionine load, recovery of enzyme activity was not complete within 7 days. In the patients receiving a methionine load, the kinetics of inactivation of methionine synthase were similar, but the rate and extent of enzyme recovery was higher than in patients not receiving methionine, and in four patients, the enzyme activity even exceeded the preoperative level. The inactivation of methionine synthase was associated with a transient increase in plasma homocysteine, and the homocysteine concentration was still increased (mean 28.7%) 7 days after anesthesia in the patients not receiving methionine. A marked peak in homocysteine concentration was observed immediately after anesthesia in the methionine-loaded patients, but the homocysteine level was still increased (mean of 30.5%) after 7 days.

The activity of the other cobalamin-dependent enzyme, methylmalonyl coenzyme A mutase, in the mononuclear white blood cells, and the serum concentration of the cobalamin marker methylmalonic acid, were not altered after nitrous oxide anesthesia or methionine loading or both.

Conclusions: Our data suggest that short time exposure to nitrous oxide selectively impairs the function of the cobalamin-dependent methionine synthase. Furthermore, preoperative administration of methionine should be considered as a means to counteract adverse effects of nitrous oxide. (Key words: Anesthetics, gases: nitrous oxide. Metabolism, cobalamin: inactivation.)

PROLONGED exposure to nitrous oxide may cause adverse effects on both the hematologic and nervous systems in humans, and the clinical symptoms and signs resemble those observed in cobalamin deficiency.¹ The target of nitrous oxide is the cobalamin-dependent enzyme methionine synthase (N⁵-methyltetrahydrofolate-homocysteine methyltransferase, EC 2.1.1.13.). Nitrous oxide probably acts by intercepting enzyme-bound monovalent cobalamin that is transiently formed during catalytic turnover. Monovalent cobalamin is oxidized to divalent cobalamin, leading to irreversible inactivation of the enzyme² (fig. 1).

Methionine synthase acts at the point of convergence between homocysteine and folate metabolism, where homocysteine is remethylated to methionine, and 5-methyltetrahydrofolate is demethylated to tetrahydrofolate (fig. 1).³ Therefore, impaired function of this enzyme causes both accumulation of homocysteine and trapping of reduced folates as 5-methyltetrahydrofolate.⁴ Both metabolic effects may play a role in pathogenesis of cobalamin deficiency.² In addition, increased concentration of plasma homocysteine is an early and sensitive indicator of cobalamin inactivation induced by nitrous oxide exposure in patients.⁵

Both the rate and extent of methionine synthase inactivation by nitrous oxide in human cells are decreased

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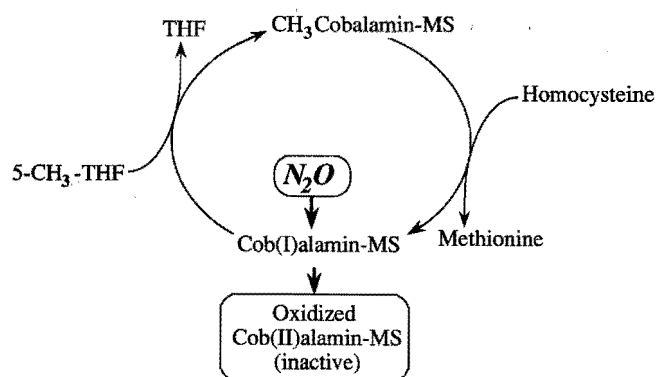


Fig. 1. Inactivation of the cobalamin-dependent enzyme methionine synthase by nitrous oxide. Cob(I)alamin = monovalent cobalamin; cob(II)alamin = divalent cobalamin; 5-CH₃-THF = 5-methyltetrahydrofolate; MS = methionine synthase; THF = tetrahydrofolate.

when the cells are cultured in the presence of low folate⁶ or high methionine⁷ concentration in the medium. Low medium folate decreases the availability of the substrate 5-methyltetrahydrofolate,⁸ and high medium methionine may increase the level of intracellular methionine causing product inhibition. Both conditions may decrease the catalytic turnover of the enzyme, which is a possible determinant of nitrous oxide-induced inactivation.⁹

Methionine loading is, in contrast to folate depletion, a practical intervention in the clinical setting, and is usually carried out to diagnose defects in homocysteine catabolism. After a standard methionine dose of 100 mg/kg, plasma methionine reaches concentrations of several hundred micromolar within 30 min, followed by a homocysteine response which peaks after 4–6 h.¹⁰

In the current study, we evaluated preoperative methionine loading as a means of protecting against cobalamin inactivation in patients receiving nitrous oxide anesthesia. We measured the effect on methionine synthase in isolated mononuclear blood cells from these patients, and the accompanying homocysteine response in plasma. In addition, we also determined whether nitrous oxide may have remote effects on the other cobalamin-dependent enzyme, methylmalonyl coenzyme A (CoA) mutase, by measuring the activity in mononuclear cells and the serum concentration of methylmalonic acid. These effects were investigated because prolonged exposure to nitrous oxide has been shown to decrease methylmalonyl CoA mutase in the liver of rats.¹¹

Materials and Methods

Chemicals

L-Methionine, DL-homocysteine, cyanocobalamin, DL-5-methyltetrahydrofolate (barium salt), methylmalonyl CoA, succinyl CoA, methylmalonic acid, and dithioerythritol were purchased from Sigma Chemical (St. Louis, MO). 2-Mercaptoethanol (pro analysis) and methanol (gradient grade) were from Merck (Darmstadt, Germany) and sodium borohydride from Fluka Chemie, AG (Switzerland). (±)-L-N⁵-[methyl-¹⁴C]-methyltetrahydrofolate (54 mCi/mmol, barium salt) was purchased from Amersham (Buckinghamshire, England) dissolved in 10 mM ascorbic acid and stored as 200- μ l aliquots in nitrogen at -80°C until use. AG 1-X8 resin, 200–400 mesh, chloride form, was purchased from Bio-Rad Laboratories (Richmond, CA). The material was slurry-packed in 5-ml bed volume polypropylene columns (4.5 \times 1.1 cm ID) obtained from Pierce (Rockford, IL), and the material was covered with a polyethylene disc with pore size of 45 μ m.

Patients and Subjects

Fourteen ASA physical status 1 or 2¹² patients (6 women and 8 men) of mean age 44 yr (range 23–68 yr) were included in the study. They underwent otolaryngologic surgery.

We also investigated the response to methionine loading in three healthy subjects (one woman and two men) aged 27–32 yr, not receiving anesthesia.

Anesthesia was induced with thiopental (5–7 mg/kg) and fentanyl (2–3 μ g/kg). Neuromuscular blockade was achieved with succinylcholine (1 mg/kg), and the tracheae intubated. For additional muscle relaxation, pancuronium (0.1 mg/kg) was used. Anesthesia was maintained with 70% nitrous oxide in 30% oxygen and isoflurane (0.5–1%), and lasted for 75–230 min. Mean duration of nitrous oxide exposure was 162 (SD 42) min in the control group (patients receiving orange juice) and 150 (SD 60) min in the methionine-loaded group.

Glycopyrrolate/neostigmine was administered to reverse the residual effect of pancuronium, and all patients received ketobemidone intravenously and paracetamol suppositories for postoperative pain relief. All patients, except patient 10, were free of persistent nausea or vomiting and had normal food intake the first and the following postoperative days.

The protocol was approved by the regional ethical committee. All patients received oral and written in-

formation about the intent of the study, possible risks and their right to withdraw from the study. Then an informed consent was signed.

Protocol

To test the possible effect of methionine loading on the inhibition of homocysteine remethylation induced by nitrous oxide, 14 patients undergoing nitrous oxide anesthesia were recruited to the study. Patients either received preoperative methionine (100 mg/kg methionine in 150 ml orange juice 2 h before anesthesia) or no methionine (150 ml orange juice); the latter patients served as controls. The beverages were administered ice cold to obtain rapid emptying of the stomach.

The patients were allocated to the two groups, each including seven subjects, according to a prerandomized list unknown to the investigator recruiting the patients. This sample size gives significance (by the Mann-Whitney rank-sum test) at the level of $P < 0.05$ with one atypical response, irrespective of its magnitude.¹³

Fasting blood samples were taken immediately before methionine loading, *i.e.*, about 2 h before start of nitrous oxide anesthesia, then immediately before start and immediately after the nitrous oxide exposure. Fasting blood samples were thereafter collected in the morning on the 1st, 2nd, 3rd, 5th, and 7th postoperative days. A similar protocol was followed for the three subjects receiving methionine alone.

In the fasting blood samples obtained from the patients before start of anesthesia, we measured hemoglobin, erythrocyte folate, serum folate, serum cobalamin, serum creatinine, total plasma homocysteine, serum methylmalonic acid, and the activities of methionine synthase and methylmalonyl CoA mutase in mononuclear white blood cells.

Blood Sampling and Processing

For the preparation of serum, venous blood (5 ml) was drawn into an evacuated blood collection tube (Vacutainer, Becton Dickinson, Rutherford, NJ) without anticoagulant. After 30 min at room temperature, the contents of each tube were centrifuged, and the serum fraction aspirated and stored at -80°C until analysis.

For the preparation of plasma and peripheral mononuclear cells, 40 ml venous blood was collected into Vacutainers containing EDTA, and immediately placed on ice. Plasma was obtained from 3 ml whole blood

by centrifugation within 30 min, and the plasma fraction stored at -80°C until analysis.

Preparation of Peripheral Mononuclear Blood Cells

From the remaining 37 ml whole blood, mononuclear white blood cells were isolated according to the method of Böyum.¹⁴ This method is based on density gradient centrifugation in a medium containing sodium metrizoate and Ficoll. The isolated fraction includes lymphocytes, monocytes, and macrophages. After gently washing with phosphate-buffered saline, the cells were counted in a Coulter counter (model 2M, Coulter Electronics, Luton, UK). The final suspension was equally divided into four tubes which were centrifuged. The cell pellets were stored at -80°C until enzyme analyses, which were performed within 2 weeks.

Analytical Methods

Methionine synthase in extracts from mononuclear cells was assayed by a slight modification¹⁵ of the radioisotope method constructed by Weissbach *et al.*¹⁶

Methylmalonyl CoA mutase activity in mononuclear cells was determined as described by Kikuchi *et al.*,¹⁷ except that the buffer concentration of the mobile phase was increased to 100 mM NaH_2PO_4 to obtain higher plate number, and thereby better resolution of methylmalonyl CoA (retention time 3.8 min) and succinyl CoA (retention time 5.7 min).

Total plasma homocysteine was determined by an automated high-performance liquid chromatography assay.¹⁸ The term "total homocysteine" refers to all homocysteine species in plasma, including protein-bound homocysteine. Methylmalonic acid in serum was assayed by a method based on high-performance liquid chromatography and fluorescence detection.¹⁹ Plasma methionine was determined in deproteinized plasma with an assay based on derivatization with *o*-phthalaldehyde and fluorescence detection.²⁰

Serum cobalamin was determined with a microparticle enzyme intrinsic factor assay run on an IMx system (Abbot, Abbott Park, IL). Serum folate and erythrocyte folate were assayed using the Quantaphase folate radioassay produced by Bio Rad (Hercules, CA).

Statistical Analyses

The small number of patients in the analysis groups did not allow testing for normal distribution of values, and therefore nonparametric tests were used.

The difference in methionine synthase or total plasma homocysteine between men and women was tested for using the Mann-Whitney rank-sum test. The time-related change in these values was evaluated using Friedman's test (nonparametric analysis of variance). When significant changes were found, comparisons of these parameters within the same group of patients were performed using Wilcoxon's matched-pair signed-rank test. Differences in values between control patients and patients subjected to a methionine load were evaluated using the Mann-Whitney rank-sum test. Correlation between parameters were tested using Spearman's rank correlation coefficient (r_s). The P -values are given as two-tailed, and $P < 0.05$ is regarded as significant.

Results

Preoperative Blood Values

All patients included had values of erythrocyte folate, serum folate, serum cobalamin, and serum creatinine within the reference intervals. Mean total plasma homocysteine was 9.2 (range 5.2–13.1) μM ; mean serum methylmalonic acid was 0.07 (range 0.02–0.11) μM ; and mean plasma methionine was 25.6 (range 13.7–33.6) μM (table 1). There was no significant difference in any of these parameters between the methionine-loaded and control patients, and in methionine synthase or total homocysteine between men ($n = 8$) and women ($n = 6$). Serum folate ($r_s = -0.73$, $P < 0.005$) and serum cobalamin ($r_s = -0.61$, $P < 0.05$) were negatively correlated to total plasma homocysteine. There was a trend toward a negative correlation ($r_s = -0.52$, $P < 0.1$) between erythrocyte folate and total plasma homocysteine, but there was no correlation between serum concentrations of either vitamin and serum methylmalonic acid ($P = 0.5$).

Methionine Synthase

In both methionine-loaded and control patients, methionine synthase in white blood cells was rapidly inactivated after nitrous oxide exposure reaching a nadir ranging from 18–68% of preoperative values. These low activities were measured between end of anesthesia and the second postoperative morning, and the kinetics were similar in the two groups (figs. 2, left and 3, left). The nadir as a percentage of preoperative value was 42% (SD 15%) in the control group, and 40% (SD 10%) in patients receiving methionine (fig. 4). There was a significant ($P < 0.02$ at time 24 h) decline in methi-

onine synthase in both groups, which was not different for the two groups ($P > 0.5$).

The enzyme recovery was characterized by an initial rapid phase lasting for 2–3 days and leveling off in most patients (figs. 2, left and 3, left). In the control group, the activity of methionine synthase had not returned to preoperative level in any patient within 7 days (fig. 2, left). In contrast, enzyme recovery was faster in the patients receiving methionine, and the activity equaled (three patients) or even exceeded (four patients) the preoperative activity within 5–7 days (fig. 3, left). The values given as percentage of preoperative levels were significantly ($P < 0.03$) different between the groups at 5 and 7 days (fig. 4). When the statistical evaluation was based on the absolute change relative to the preoperative enzyme activity, significant ($P = 0.03$) difference was observed at time 7 days.

No consistent pattern in methionine synthase activity was observed in mononuclear blood cells from 3 healthy subjects after receiving a standard methionine load (fig. 5).

Total Plasma Homocysteine and Plasma Methionine

The relative changes in plasma methionine, total plasma homocysteine and white blood cell methionine synthase, given as percentage of preoperative values, are shown in figure 4.

We found no significant decrease in plasma methionine after nitrous oxide exposure in control patients. Recently, we reported that nitrous oxide exposure lasting for 70–720 min reduced plasma methionine concentration. The change occurred after 3–6 h, and the concentration returned to normal after 24 h.⁵ Thus, the current study was not optimized to detect possible changes in plasma methionine concentration.

In control patients, total plasma homocysteine showed a significant ($P \leq 0.03$) increase after nitrous oxide anesthesia. The plasma profile for total homocysteine was different between the patients in that the concentration peaked after 12–36 h and then declined in four patients (patients 1–4), whereas it showed a progressive increase for at least 5–7 days in three others (patients 5–7). The concentration did not return to the preoperative value in any patient after 7 days (in patient 5, not after 5 days; fig. 2, right), and at this time point, the mean increase was 28.7% (SD 8.9%) (fig. 4), which was significantly ($P = 0.03$) higher than values measured before nitrous oxide anesthesia.

Table 1. Patient Characteristics and Preoperative Blood Values

Patient No.	Age (yr)	Sex	Anesthesia (min)	Hb (g/L)	cbl (pM)	sF (nM)	eF (nM)	Creat (μ M)	Met (μ M)	MS (U)*	Hcy (μ M)	MMA Mutase (U)†	MMA (μ M)
Patients not receiving methionine													
1	56	M	230	14.7	381	17.0	875	76	26.5	0.34	10.6	1.12	0.02
2	34	M	156	14.7	401	10.5	409	92	17.6	0.03	11.9	ND	0.06
3	27	F	198	13.8	656	24.9	704	77	26.1	0.30	5.2	1.73	0.04
4	24	M	156	17.6	458	19.3	537	80	28.2	0.17	6.8	1.50	0.04
5	64	F	186	13.6	313	25.7	959	82	33.6	0.20	5.2	ND	0.10
6	55	M	120	14.7	353	14.6	404	96	25.6	0.14	10.2	0.92	0.07
7	54	M	102	15.2	190	7.4	542	88	25.6	0.19	12.2	1.38	0.11
Mean \pm SD			164 \pm 45	14.9 \pm 1.3	393 \pm 143	17.1 \pm 6.9	633 \pm 220	84.4 \pm 7.7	26.2 \pm 4.4	0.20 \pm 0.10	8.9 \pm 3.1	1.33 \pm 0.32	0.06 \pm 0.03
Patients undergoing methionine loading													
8	56	M	210	13.5	258	8.1	409	ND	27.1	0.06	13.1	0.58	0.05
9	55	F	222	12.2	616	17.3	886	64	26.8	0.35	5.2	1.54	0.11
10	68	F	84	12.7	235	17.5	724	82	24.4	0.19	12.0	1.19	0.05
11	30	M	132	15.4	428	13.2	880	92	28.4	0.23	7.0	0.93	0.10
12	23	M	180	13.2	476	9.0	322	95	25.4	0.08	10.6	1.56	0.07
13	44	F	162	12.0	361	9.3	593	71	13.7	0.19	9.9	0.91	0.05
14	25	F	75	12.6	359	21.0	479	66	28.8	0.20	9.4	1.33	0.09
Mean \pm SD			152 \pm 58	13.1 \pm 1.2	390 \pm 131	13.6 \pm 5.1	613 \pm 225	78.3 \pm 13.3	24.9 \pm 4.8	0.19 \pm 0.10	9.6 \pm 2.8	1.16 \pm 0.36	0.07 \pm 0.03

Hb: hemoglobin; cbl: cobalamin; sF: serum folate; eF: erythrocyte folate; Creat: creatinine; Met: methionine; MS: methionine synthase; Hcy: total homocysteine; MMA mutase; methylmalonyl CoA mutase; MMA: serum methylmalonic acid; M: male; F: female; ND: not determined.

Normal ranges: hemoglobin, 11.6–16.6 g/100 ml; serum cobalamin, 150–840 pM; serum folate, >5.7 nM; erythrocyte folate, >230 nM; serum creatinine, 55–120 μ M; plasma methionine, 10–55 μ M; total plasma homocysteine, 5–15 μ M; serum methylmalonic acid, <0.340 μ M.

* Units given as nmol/h per 10^6 cells.

† Units given as nmol \cdot min⁻¹ \cdot mg⁻¹ protein.

NITROUS OXIDE AND COBALAMIN FUNCTION

In the patients subjected to methionine loading, there was a marked, transient increase in both plasma methionine (fig. 4, top) and total homocysteine (fig. 3, right and fig. 4, bottom). This response to methionine loading confirms published data.^{21,22}

Plasma methionine returned to normal within 24–36 h, whereas total homocysteine more slowly approached preoperative concentration, which was reached within 7 days in two patients (patients 11 and 14). At this time point, the mean increase was 30.5% (SD 21.6%) (fig. 4, bottom), which is not significantly different ($P \geq 0.5$) from the increase in control patients. The statistical evaluation was done on both percentage values and absolute change relative to preoperative concentrations.

Plasma homocysteine reached pretreatment concentrations within 48–78 h in the three subjects not exposed to nitrous oxide (fig. 5).

Methylmalonyl CoA Mutase and Serum Methylmalonic Acid

The preoperative enzyme activities and serum methylmalonic acid concentrations are listed in table 1. No significant change in these parameters was observed after nitrous oxide anesthesia or after methionine loading combined with nitrous oxide anesthesia (data not shown).

Discussion

Study Design and Parameters Investigated

The aim of the current work was to investigate whether preoperative methionine loading influences the effects of nitrous oxide on methionine synthase activity and plasma homocysteine. Therefore, both study groups received nitrous oxide, and a third control group not receiving nitrous oxide was not included. In principle, this design does not distinguish between effects from isoflurane and nitrous oxide. However, anesthesia with isoflurane or enflurane, in contrast to nitrous oxide anesthesia, does not inactivate methionine synthase in human liver²³ or placenta,²⁴ and total intravenous anesthesia without nitrous oxide has no effect on plasma homocysteine.⁵

We measured methionine synthase in isolated mononuclear white blood cells. This source of enzyme was chosen for practical reasons, since it allows repeated sampling necessary for kinetic studies. The published studies on the inactivation of human methionine synthase are based on measurements of enzyme activity in

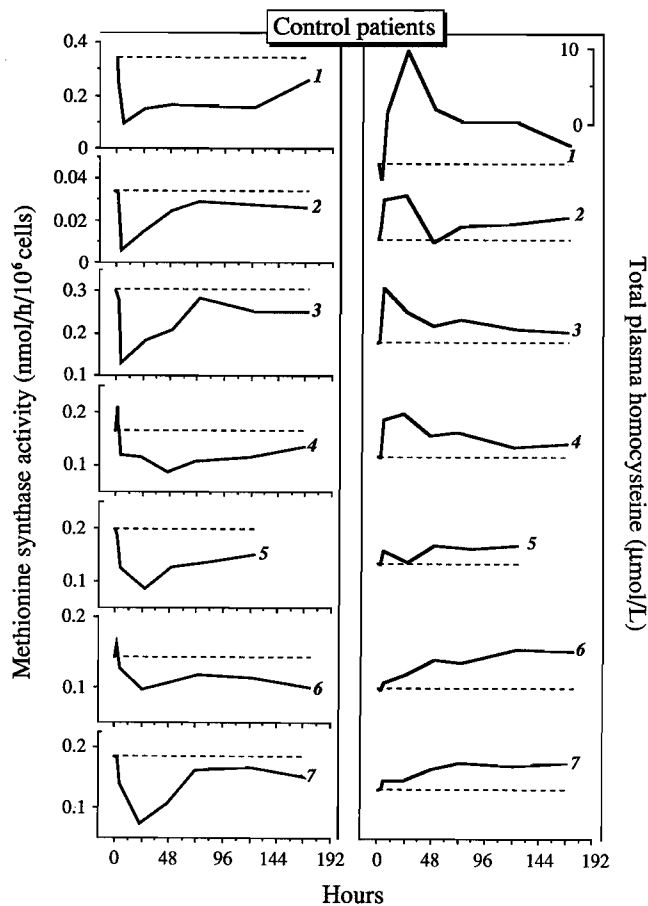


Fig. 2. Methionine synthase activity (*left*) and total plasma homocysteine (*right*) in control patients after nitrous oxide exposure. The time scale starts 2 h before induction of anaesthesia. Further experimental details are given in text. Patients are identified by numbers, which correspond to those listed in table 1.

tissue samples obtained during surgery^{23,24} or from placenta after delivery,²⁵ and are limited to single time-point determinations.

A reservation against white blood cells as enzyme source should be made, since methionine synthase in these cells may respond differently to nitrous oxide compared to the enzyme from other tissues or cells. This possibility should be considered, especially because the inactivation induced by nitrous oxide is related to catalytic turnover,⁹ which is probably low in human lymphocytes.²⁶ Furthermore, folate status in mononuclear cells may change independently of whole body folate status,²⁷ and cellular folate content seems to be a critical factor for the response of methionine synthase to nitrous oxide.⁶

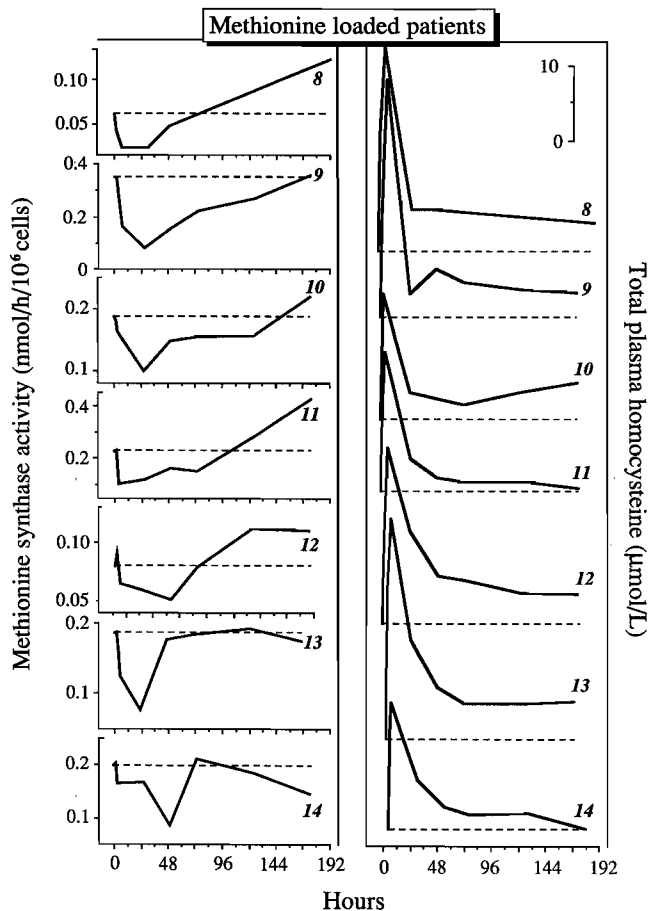


Fig. 3. Methionine synthase activity (*left*) and total plasma homocysteine (*right*) in patients undergoing methionine loading. The methionine loading started 2 h before induction of anaesthesia. Further experimental details are given in text. Patients are identified by numbers, which correspond to those listed in table 1.

The specific activity of methionine synthase in mononuclear white blood reported by us (mean 0.2 [SD 0.1] $\text{nmol} \cdot \text{h}^{-1} \cdot 10^{-6}$ cells; table 1) equals the activity determined by others,²⁸⁻³⁰ but is about 10-fold greater than the activity found in one study.³¹ We found large variations in the activity between subjects (table 1). Others have also reported large variations in methionine synthase activity in human lymphocytes, with standard deviation approaching²⁹ or exceeding³¹ one half of the mean value. Methionine synthase in human lymphocytes is enhanced during cell division³² and mitogen stimulation.³³ This suggests that the individual variations in enzyme activity may be related to the proliferative sequence of lymphocytes.³⁰ However, repeated measurements of enzyme activity in different

blood samples from the same subjects showed small variations²⁹ (fig. 5).

Total plasma homocysteine is a sensitive indicator of impaired function of cobalamin and folate and probably reflects whole body folate or cobalamin status.³⁴

Previously, we have shown that after a methionine load, plasma homocysteine returns to baseline within

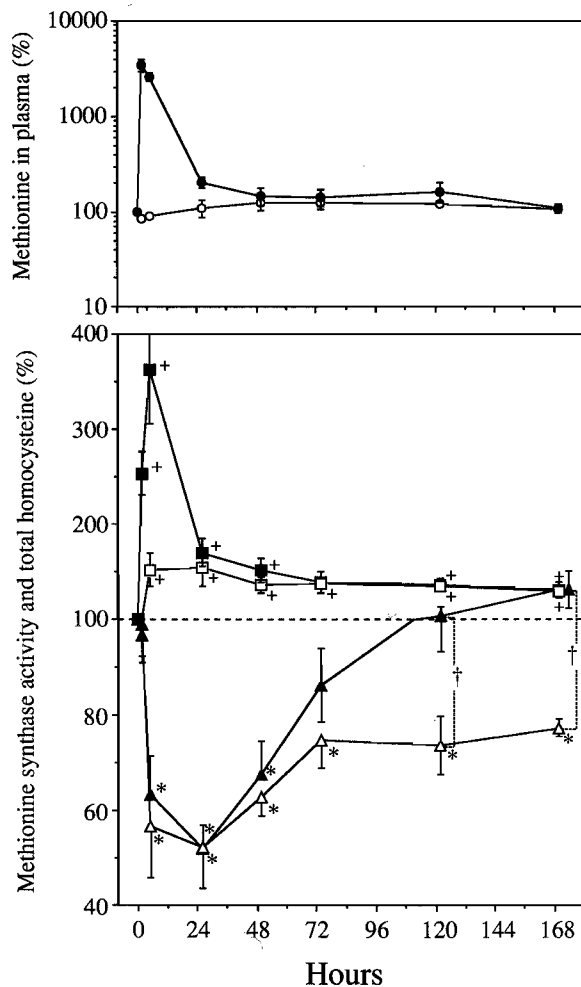


Fig. 4. The average changes in plasma methionine (*top*), methionine synthase, and total homocysteine (*bottom*) in control patients (open symbols) and in patients subjected to methionine loading (closed symbols). The time scale (x-axis) is the same as in figures 2 and 3. Data are presented as mean ($n = 7$) percentage of preoperative values \pm standard error of mean. Circles = plasma methionine; triangles = methionine synthase; squares = total plasma homocysteine. * $P \leq 0.03$, methionine synthase activity versus the preoperative activity by Wilcoxon's matched-pair signed-rank test; † $P \leq 0.03$, total plasma homocysteine versus the preoperative values by Wilcoxon's matched-pair signed-rank test; ‡ $P \leq 0.03$, percentage change in methionine synthase activity in methionine-loaded patients versus controls by the Mann-Whitney rank-sum test.

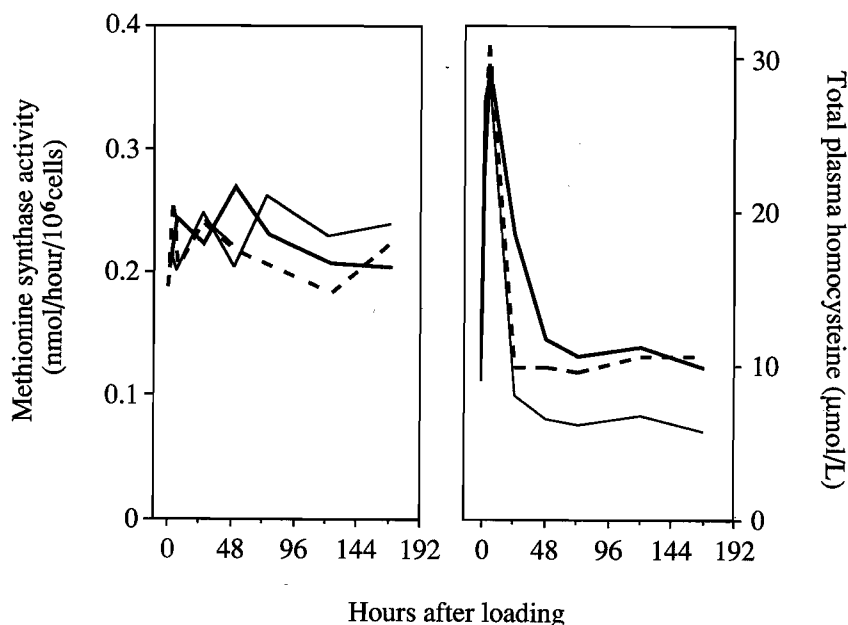


Fig. 5. Methionine synthase activity (*left*) and total plasma homocysteine (*right*) in three healthy subjects undergoing methionine loading. They were not exposed to nitrous oxide.

2–3 days in healthy persons,^{21,22} and this is confirmed by the data presented in figure 5. In contrast, the nitrous oxide induced effect on plasma homocysteine persists for at least 7 days.⁵ Thus, total plasma homocysteine determined in the time interval between 3 and 7 days after methionine loading and anesthesia may reflect the effect of nitrous oxide on whole-body cobalamin status.

The activity of methylmalonyl CoA mutase in mononuclear white blood cells and serum methylmalonic acid were measured to investigate possible remote effects on this cobalamin-dependent system. In the current study, no changes in these parameters were observed, which is in accordance with the observation in rats showing that nitrous oxide exposure for longer than 48 h is required to inhibit liver methylmalonyl CoA mutase.¹¹

Inhibition of Homocysteine Remethylation

Nitrous oxide anesthesia inhibited homocysteine remethylation in our patients. This was demonstrated by the marked reduction in methionine synthase activity in white blood cells and the concomitant increase in plasma homocysteine (figs. 2–4).

The duration of nitrous oxide exposure was 75–230 min. Notably, in 10 of 14 patients, the methionine synthase activity in circulating mononuclear cells reached a nadir 24–48 h later (figs. 2 and 3), suggesting that the effect of nitrous oxide outlasts the duration of anesthesia. However, nitrous oxide is believed to directly

interact with the enzyme, and there is no experimental data showing delayed inactivation of methionine synthase. A possible explanation is related to recirculation of lymphocytes between organs and homing of lymphocytes to restricted anatomic sites, including lung nodes.³⁵ It is conceivable that lymphocytes residing in the latter location are exposed to particularly high concentrations of nitrous oxide, resulting in extensive inactivation of methionine synthase in certain lymphocyte subpopulations. Notably, lymphocytes that do not recognize cognate antigen exit the lymphoid organ and recirculate within about 24 h.³⁶

In every patient not receiving methionine, the decrease in methionine synthase activity was followed by a slow recovery of enzyme activity, and there was a concurrent return of plasma homocysteine toward preoperative concentrations in four patients (fig. 2). During the recovery period, no strict correlation was observed between the alterations in enzyme activity in white blood cells and the changes in plasma homocysteine. The disparity between enzyme activity and the plasma homocysteine profile in three patients (patients 5–7; fig. 2) may point to a differential response of the enzyme in white blood cells versus other tissues.

Effect of Methionine Loading

Total homocysteine was normalized within 7 days in only two patients (patients 11 and 14; fig. 3). Notably, both were subjected to methionine loading. Methionine

loading did apparently not affect the rate and extent of inactivation of methionine synthase in white blood cells, but it significantly enhanced the recovery of the enzyme (fig. 3). This unexpected finding is in contrast to the observation made with cultured proliferating human fibroblasts exposed to nitrous oxide. In cultured cells, methionine decreases the initial rate of inactivation by nitrous oxide.⁷

Different response *in vitro*⁷ and in patients may be related to the different cell types investigated, or to study design. The cultured cells were continuously exposed to nitrous oxide and various concentrations of methionine for 48 h,⁷ whereas the patients were exposed sequentially to methionine followed by nitrous oxide for less than 4 h.

Possible Mechanisms

High concentrations of methionine may enhance enzyme recovery in patients by promoting enzyme reactivation or new synthesis of enzyme. The slow time course (figs. 3 and 4) is consistent with both mechanisms.

Experiments with isolated enzyme suggest that nitrous oxide induced inactivation is an irreversible process,³⁷ suggesting that new synthesis of enzyme is required for recovery of enzyme activity. However, excess methionine suppresses methionine synthase in cultured baby hamster kidney cells³⁸ and rat liver,³⁹ and methionine loading alone did not affect white blood cell methionine synthase in healthy subjects (fig. 5). These observations do not support the possibility that methionine enhances the synthesis of methionine synthase.

A model for the enhanced restoration of methionine synthase by methionine (figs. 3 and 4) is based on the idea that lymphocyte subpopulations are exposed to different concentrations of nitrous oxide or methionine, or that the response to methionine of various lymphocyte populations is different. Thus, the rate of recovery of methionine synthase in white blood cells may reflect the kinetics of lymphocyte recirculation.³⁶

Plasma homocysteine reached the preoperative value within 7 days in only two (patients 11 and 14) of seven patients receiving nitrous oxide plus methionine (fig. 3). This contrasts to the homocysteine response after a methionine load in healthy subjects where plasma homocysteine is normal within 2–3 days^{21,22} (fig. 5). Notably, rapid return of plasma homocysteine after a

methionine load is also observed in psoriasis patients receiving low dose of methotrexate, which causes an indirect inhibition of methionine synthase.⁴⁰ Thus, prolonged elevation of plasma homocysteine may reflect remaining inactivation of methionine synthase in some tissues.

A possible explanation is based on the assumption that a methionine load protects methionine synthase in some but not all tissues or cells. The liver is responsible for about 75% of the overall transmethylation and thereby the total homocysteine production.⁴¹ It is observed that methionine synthase in isolated rat liver cells is not protected against nitrous oxide-induced inactivation by high concentrations of methionine.[#] No protection of the liver enzyme may explain that methionine loading did not enhance the return of plasma homocysteine toward normal within 7 days (figs. 3 and 4).

Clinical Implications

Means to counteract cobalamin inactivation may become clinically useful in patients who are particularly susceptible to nitrous oxide toxicity, for example the debilitated or cobalamin-deficient patients undergoing prolonged anesthesia.⁴² Methionine administration protects monkeys⁴³ or fruit bats⁴⁴ against neurologic impairment caused by nitrous oxide, and there is a recent case report on arrest of progression and accelerated recovery from myeloneuropathy in one patient treated with methionine after recreational misuse of nitrous oxide.⁴⁵ The current investigation suggests that methionine loading may restore the methionine synthase function in patients undergoing nitrous oxide anesthesia, but interpretation of the kinetics of inactivation and recovery of methionine synthase in mononuclear white cells may be complicated by the homing and recirculation of lymphocytes.³⁵ Results of the current study should motivate long-term follow-up studies on methionine prophylaxis in selected patients receiving prolonged nitrous oxide anesthesia.

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