EFFECT OF SHORT-TERM ADMINISTRATION OF NITROUS OXIDE ON PLASMA CONCENTRATIONS OF METHIONINE, TRYPTOPHAN, PHENYLALANINE AND S-ADENOSYL METHIONINE IN MAN

J. F. NUNN, N. M. SHARER, T. BOTTIGLIERI AND J. ROSSITER

There is now ample evidence that nitrous oxide causes inactivation of methionine synthase in man and rodents (Deacon et al., 1980; Koblin et al., 1981; Koblin et al., 1982), the onset of inhibition being apparently much slower in man than in the rodent. The resultant change in plasma methionine concentration is difficult to predict, since it depends on many factors. We have shown, however, that the plasma methionine concentration decreases from a mean preoperative value of 20 μmol litre⁻¹ to 6 μmol litre⁻¹ following an administration of nitrous oxide lasting 8 h (Skacel et al., 1983). No information is available following administrations of shorter duration and there have been no measurements in rodents for exposures lasting less than 24 h. Thus, there are no data on the changes in methionine concentration during exposure to nitrous oxide for durations which are relevant to most routine surgical procedures. Not only is the plasma methionine concentration important in its own right, but it provides a relatively non-invasive index of the inhibition of methionine synthase, although it is influenced by the other factors discussed below.

We have, therefore, investigated the changes in the serum concentration of methionine following routine anaesthetics in which the administration of nitrous oxide lasted between 25 and 217 min. In addition, preoperative methionine concentrations (in patients) have been compared with values obtained in control subjects. Our analytical technique also indicated concentrations of tryptophan and phenylalanine and we have used these for comparisons between methionine, which is directly affected by methionine synthase, and other amino acids which are not so affected. We also measured the plasma concentration of S-adenosyl methionine (SAM), a major intermediate metabolite of methionine, which has not previously been measured in man in relation to the administration of nitrous oxide.

SUMMARY

Plasma concentrations of methionine, tryptophan, phenylalanine and S-adenosyl methionine (SAM) were measured at the beginning and end of general anaesthesia (for routine surgery) lasting between 25 and 217 min which included 60–70% nitrous oxide. Plasma methionine, tryptophan and phenylalanine concentrations in patients prepared for surgery averaged 68 (SD 11.3)% of values in a group of normal control subjects. At the end of administration of nitrous oxide (mean duration 88 min) there were no significant changes in the mean methionine and phenylalanine concentrations, but the mean concentration of tryptophan was reduced by 15% (P = 0.0075). SAM concentrations averaged 40% greater in males than females in all groups, but there were no significant differences between the concentrations in the control subjects and the patients before or after the administration of nitrous oxide.

PATIENTS, SUBJECTS AND METHODS

Patients and subjects

A series of 16 patients scheduled for routine surgery were selected at random and 5-ml blood
samples were withdrawn immediately before commencing the administration of the nitrous oxide. Further samples were taken within 5 min of the end of the administration of the nitrous oxide. Since the samples were withdrawn from a cannula already in position, the Hospital Ethics Committee approved the project as a minor procedure not requiring informed consent. Details of the patients are presented in table I. All patients were scheduled for surgery in the morning and had taken nothing by mouth since midnight. All patients except GW had normal nutritional intake up to the day before surgery. Only Hartmann's solution was administered by i.v. infusion during the operation, but GW received 5% dextrose solution 1 litre during the night before surgery. One patient (VD) received one unit of blood during surgery and the methionine content of this blood was 28.3 μmol litre⁻¹. In four other patients, additional samples were withdrawn during the early postoperative period.

The anaesthetic technique was selected by the anaesthetist, but all patients received nitrous oxide in a concentration within the range 60–70%.
Other drugs administered are listed in table I. All patients who received the neuromuscular blocking agents alcuronium, pancuronium, atracurium or tubocurarine were ventilated artificially, the remainder being allowed to breathe spontaneously. Premedication was with an opioid and an antialagogue. No drug shown in table I—other than nitrous oxide—is known to affect vitamin B12.

The patients were compared with a group of 16 staff members who had taken their normal breakfast (table II). They donated samples of venous blood during the morning, within the same time limits (08.44 h-13.00 h) as those during which the preoperative samples from the patients were obtained. All samples were taken into heparin and stored on ice.

**Amino acid analysis**

Blood samples were analysed for methionine, tryptophan and phenylalanine concentrations using high pressure liquid chromatography (HPLC). The blood was centrifuged at 1500 g for 10 min at 4 °C and the plasma removed. One volume of serum was deproteinized with four volumes of methanol and left to stand at 4 °C for 30 min. The precipitate was spun down and the supernatant stored at −20 °C until it was analysed on a 25-cm column of Hypersil ODS (5 µm) (self-packed) and sodium potassium phosphate buffer 0.1 mol litre⁻¹, pH 7.0 with 50% methanol (HPLC grade, Rathburn Chemicals Ltd) and EDTA solution 10 mg dl⁻¹ as the isocratic solvent. A Pye Unicam L33-XP pump was used at a flow rate of 1 ml min⁻¹. Samples were introduced using a 7120 Rheodyne injection valve with a 100-µl loop. An EDT LDA 15 electrochemical detector was used with a glassy carbon electrode and a 0.75-V oxidative current at a sensitivity of 100 nA. Output was recorded on a Philips PM 8251 chart recorder. Elution time for methionine was 14 min. The limit of detection was 2 µmol litre⁻¹ and the coefficient of variation for replicate samples was 7.4%.

Plasma samples and standards were derivatized with o-phthalaldehyde (OPT)/mercaptoethanol (2ME) (Joseph and Davies, 1982). A sample volume of 40 µl was reacted with 200 µl of OPT reagent (containing OPT 27 mg in 5 ml of disodium tetraborate 0.1 mol litre⁻¹ with 2ME 20 µl added) for 2 min. Amino acid standard solutions were made up using chromatographically pure amino acids (Sigma). Recovery of standards added to plasma was 95–100 % and the yield of the reaction was estimated at 100% using radio-labelled methionine.

Peak heights obtained from each sample were quantified from the linear calibration curves previously constructed and reconfirmed at the time of each analysis.

**S-adenosyl methionine (SAM)**

Plasma concentrations of SAM were estimated by a highly sensitive enzymatic double isotope method (Baldessarini and Kobin, 1966). Plasma samples were prepared, immediately deproteinized with perchloric acid 0.8 mol litre⁻¹ and centrifuged.
TABLE III. Plasma concentrations of amino acids and S-adenosyl methionine (SAM) (mean (SD)). *Excludes value for patient HB whose SAM concentration was 2.4 SD above the mean both before and after nitrous oxide. †n = 6

<table>
<thead>
<tr>
<th></th>
<th>Methionine (μmol litre^-1)</th>
<th>Tryptophan (μmol litre^-1)</th>
<th>Phenylalanine (μmol litre^-1)</th>
<th>S-adenosyl methionine (ng litre^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Control subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>21.1 (3.8)</td>
<td>24.0 (5.8)</td>
<td>67.6 (10.2)</td>
<td>59.1 (4.6)</td>
</tr>
<tr>
<td>Patients: before operation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.8 (2.1)</td>
<td>13.7 (3.4)</td>
<td>45.6 (11.5)</td>
<td>38.2 (9.8)</td>
</tr>
<tr>
<td>Patients: end of administration of nitrous oxide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.7 (2.9)</td>
<td>14.1 (2.5)</td>
<td>38.3 (8.6)</td>
<td>33.1 (7.9)</td>
</tr>
</tbody>
</table>

Mean reduction during operation
0.10 -0.48 7.31 5.06 1.19 -0.48 0.22 -0.82†

Standard error of mean reduction
1.05 0.81 3.31 2.42 3.01 2.33 1.75 1.05

Significance of reduction
ns ns ns ns ns ns ns ns

Significance of difference between controls and patients before op. (P)
0.0035 0.0007 0.0012 < 0.0001 ns < 0.0001 ns
< 0.0001 < 0.0001 < 0.0001 ns

Fig. 2. Individual changes in plasma concentrations of amino acids during exposure to nitrous oxide.

The clear supernatant was then stored at —20 °C.

The assay method is based on the dilution of endogenous SAM present in the sample with SAM (14C-methyl) as an internal standard. The mixture was incubated with the methyl acceptor N-H3-acetyl serotonin and the enzyme hydroxyindole O-methyl transferase (bovine pineal). The product (a mixture of 14C-melatonin-3H-methylacetyl and melatonin-3H-methoxy acetyl) was extracted in chloroform and the ratio of the specific activities was a function of the concentration of SAM in the original sample. Calibration of the technique was with S-adenosyl-1-methionine, generously donated by Dr G. Stramentinoli of Bioresearch Company, Milan.
Statistical methods

Data from the control subjects and the patients in the preoperative period were compared using the unpaired t test, whereas the paired t test was used to compare the pre- and post-operative results.

RESULTS

Comparison between control subjects and patients in the preoperative period

Immediately before the induction of anaesthesia, patients showed plasma concentrations of methionine, tryptophan and phenylalanine which were significantly lower than those of the control subjects. The differences were highly significant when the two sexes were considered together and also when considered separately, except in the case of the phenylalanine concentrations in males (fig. 1, table III). In contrast, SAM concentrations were not significantly different between the control subjects and the patients before anaesthesia, but showed a consistent difference between males and females with concentrations in males 40% greater than those in females (SD 9%). Methionine concentrations in the control subjects showed no relationship to the methionine content of the breakfasts which they had consumed (table II).

Comparison between preoperative and postoperative samples

There were no significant changes in the concentrations of methionine, phenylalanine and SAM (fig. 2, table III). Tryptophan concentrations were, however, decreased significantly, but only when both sexes were considered together ($P = 0.0075$). A plot of methionine concentrations against the duration of the administration of nitrous oxide showed no trend which could be related to the duration of the administration (fig. 3).

In four patients, samples were taken in the postoperative period and in all three sampled more than 1 h after surgery there were decreases in the methionine concentration (3–5 μmol litre$^{-1}$) (table IV).

The patient GW in whom there had been restricted nutritional intake before surgery showed the lowest preoperative concentrations for all three amino acids (tryptophan 24 μmol litre$^{-1}$, methionine 9.8 μmol litre$^{-1}$ and phenylalanine 35.5 μmol litre$^{-1}$), but the SAM concentration was above the mean value for female patients. Patient VD showed a small increase in methionine concentration, from 12 to 15.3 μmol litre$^{-1}$, following the administration of one unit of blood containing methionine 28.3 μmol litre$^{-1}$. 
DISCUSSION

Preoperative values

Results in the control subjects were similar to those reported in other studies both from our institute and elsewhere (Labrosse et al., 1967; Perry and Hansen, 1969; Armstrong and Stave, 1973; Fasman, 1976; Milsom, Morgan and Sherlock, 1979; Nunn et al., 1982) (table V). Samples drawn immediately before the induction of anaesthesia showed much lower values of methionine, tryptophan and phenylalanine than in our control subjects ($P < 0.0001$ for each). SAM concentrations did not differ significantly, although the concentrations in males were consistently greater than those in females.

The decreases in concentration noted following preparation for surgery accord closely with the percentage reduction in the concentrations of methionine and phenylalanine reported by Milsom and her colleagues (1979) in subjects who had fasted overnight when compared with those who had taken a light breakfast (6 kcal/kg body weight, 9% of the calories being derived from protein (Morgan, personal communication)). There was no correlation between the methionine concentrations in our control subjects and the methionine content of the breakfast which they had taken.

There is now a consensus that there is no discernible diurnal variation in plasma concentration of methionine (Milsom, Morgan and Sherlock, 1979), but there is no general agreement on whether there is a difference between male and female subjects. The mean age of the control subjects was less than that of the patients, but there was no statistical evidence of age-dependence of amino acid concentrations in the present study and it has been reported elsewhere that there is no significant relationship between the plasma concentrations of methionine, tryptophan and phenylalanine and age in adults (Armstrong and Stave, 1973; Milsom, Morgan and Sherlock, 1979).

Thus, it would appear that the overall decreases in the concentrations of all three amino acids observed in the patients before surgery were real and could be attributed to the missed breakfast. This effect far outweighs any effect of the administration of nitrous oxide, for the duration we have studied, during surgery.

Effect of administration of nitrous oxide

There are few studies of the effect of exposure
NITROUS OXIDE AND PLASMA AMINO ACID CONCENTRATIONS

Table V. Previously reported normal values for plasma amino acid concentrations (μmol litre⁻¹)

<table>
<thead>
<tr>
<th></th>
<th>Methionine</th>
<th>Tryptophan</th>
<th>Phenylalanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasman (1976)</td>
<td>22.8</td>
<td>—</td>
<td>50.9</td>
</tr>
<tr>
<td>Labrosse et al. (1967)</td>
<td>20.8</td>
<td>—</td>
<td>53.8</td>
</tr>
<tr>
<td>Perry and Hansen (1969)</td>
<td>21.0</td>
<td>31.0</td>
<td>48.0</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fastig</td>
<td>32.0</td>
<td>62.0</td>
<td>63.0</td>
</tr>
<tr>
<td>Non-fastig</td>
<td>35.9</td>
<td>—</td>
<td>76.7</td>
</tr>
<tr>
<td>Nunn et al. (1982)</td>
<td>28.7</td>
<td>—</td>
<td>57.6</td>
</tr>
<tr>
<td>This study</td>
<td>21.1</td>
<td>67.7</td>
<td>66.0</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td>77.1</td>
</tr>
<tr>
<td>Fastig</td>
<td>28.0</td>
<td>47.0</td>
<td>52.0</td>
</tr>
<tr>
<td>Non-fastig</td>
<td>26.8</td>
<td>—</td>
<td>57.6</td>
</tr>
<tr>
<td>This study</td>
<td>24.0</td>
<td>59.1</td>
<td>77.1</td>
</tr>
</tbody>
</table>

reported in an abstract that serum methionine concentration decreased significantly within the first 1 h of nitrous oxide anaesthesia and remained low at 24 h, but no figures were given.

The present study shows no evidence of change in any of the amino-acid concentrations, with the exception of tryptophan, for which the decrease approached conventional significance levels for both male (P = 0.06) and female (P = 0.08) patients and was significant when both sexes were considered together (P = 0.0075). No explanation for this is evident at present, but tryptophan is strongly bound to plasma protein and this fraction would not be measured in the analytical technique which we used.

Plasma concentrations of SAM were unchanged. We are unaware of any other data for the effect of nitrous oxide on plasma SAM concentrations in either man or rodents. However, in rats, liver SAM concentrations showed an initial increase after 12 h of exposure to 50% nitrous oxide, followed by a gradual decrease between 24 h and 8 days of exposure reaching one-third of control values (Lumb et al., 1983). Concentrations in the brain were unchanged.

Postoperative changes

Since the recovery of methionine synthase activity is slow (Deacon et al., 1980), it is possible that an "after-drop" in methionine concentration may occur after the end of an administration of nitrous oxide. Amos and his colleagues (1982) found abnormal deoxyuridine (dU) suppression tests in 85% of patients admitted to intensive care after receiving nitrous oxide for less than 2 h, and 95% of those in whom the administration lasted.
2–4 h. The dU suppression test was normal in all 15 patients who had not received nitrous oxide.

The present study was not designed to study changes in the postoperative period, but samples were taken from four patients in the recovery room (table IV). There were decreases in methionine concentrations of 3–5 \( \mu \text{mol litre}^{-1} \) in the three patients who had received nitrous oxide for more than 33 min and in whom samples were taken 2 h or more after the start of anaesthesia. Further studies are clearly required in the postoperative period.

**Time course of changes in man**

Hepatic methionine synthase activity is very rapidly inhibited by nitrous oxide in rodents (Deacon et al., 1980; Koblin et al., 1981), but the limited evidence available from liver biopsies suggests that the onset of inhibition is much slower in man. Koblin and his colleagues (1982) indicate an interpolated mean value of 36% inhibition after 88 min exposure (the mean duration of administration of nitrous oxide in this study). Kano and co-workers (1981) found an even slower onset of inhibition of methionine synthase activity in human bone marrow and these studies would accord with the normal methionine concentrations found by us.

The time course of the inhibition of methionine synthase and the development of its metabolic consequences (fig. 4) are of practical importance. Plasma methionine concentration is reduced after

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**Fig. 4.** Metabolic map linking methionine synthesis with the conversion of deoxyuridine to deoxythymidine. 5, 10-Methylene tetrahydrofolate is the obligatory carbon donor for the latter reaction. (Reproduced with permission from *Trends in Pharmacological Sciences* (Nunn, 1984).)
8 h administration of nitrous oxide and abnormal thymidine synthesis (dU suppression test) may follow administration of the same duration (Amess et al., 1978; Amos et al., 1982). A great deal of routine surgery entails the administration of nitrous oxide for periods of up to 4 h and it is not yet known what is the upper limit of duration of administration of nitrous oxide which does not result in any metabolic change attributable to the administration of nitrous oxide. The present study would seem to exclude any significant change in plasma methionine concentrations during the administration of nitrous oxide of sufficient duration for a great deal of minor and intermediate routine surgery. However, the possibility of a decrease in methionine concentration in the postoperative period is not excluded in the present study.

Factors influencing the plasma concentration of methionine

Methionine synthase activity is by no means the only factor governing the plasma concentration of methionine (fig. 5). Dietary intake is, of course, very variable, but an adequate mixed diet contains approximately 10 mmol of methionine per day. Catabolism and protein synthesis are normally in balance with a turnover of about 40 mmol of methionine per day. Utilization for other purposes such as gluconeogenesis, transulphuration reactions and, via SAM, for methylation and formylation reactions is about 20 mmol day⁻¹. The deficit between utilization and intake is made up by recycling some 10 mmol day⁻¹ via S-adenosyl homocysteine and homocysteine. In addition to the transmethylation reaction catalysed by methionine synthase (shown in fig. 4) a methyl group may be derived from betaine. This pathway is unaffected by nitrous oxide. Intravenous preparations of amino acids typically contain about 26 mmol litre⁻¹ and would presumably compensate both for the dietary loss and also for loss of methionine synthase activity.

Figure 5 shows that methionine entering the body pool from the methionine synthase reaction is only a small fraction of the total and it is therefore hardly surprising that inhibition of methionine synthase activity with nitrous oxide may have little effect in the short term on plasma methionine concentration, particularly as catabolism may be increased by surgery. In addition, it should be remembered that the utilization of methionine is intracellular, and intracellular concentrations tend to be much higher than plasma concentrations, at least in the rat, as a result of active transport (Waterlow, Garlick and Millward, 1978). Caution is therefore needed in interpretation of changes in plasma concentrations in terms of both the size of the methionine pool and its concentration at its site of utilization. For these and other reasons, further studies will be required to establish the interdependence of the time course.
of changes in methionine and SAM concentrations and the development of an abnormal deoxyuridine suppression test, indicating depletion of 5, 10-methylenetetrahydrofolate.

ACKNOWLEDGEMENTS
The authors thank Mrs Elaine Bryson for calculating the methionine content of the subjects' breakfasts and Miss Brenda Dobson for typing the manuscript. I am indebted to my surgical and anaesthetic colleagues for permission to study patients under their care.

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REFERENCES