NITROUS OXIDE AND FORMIMINOGlutAMIC ACID: EXCRETION IN SURGICAL PATIENTS AND ANAESTHETISTS

P. ARMSTRONG, P. W. H. RAE, W. M. GRAY AND A. A. SPENCE

SUMMARY

We have investigated the possible toxicity of nitrous oxide on vitamin $B_{12}$ and its sequelae upon folic acid metabolism using the urine formiminoglutamic acid excretion test, an index of the functional state of folate metabolism. Ten control subjects not exposed to nitrous oxide and five patients receiving limb surgery under local anaesthesia excreted normal amounts of formiminoglutamic acid in urine for 6 days. Fifty patients received nitrous oxide anaesthesia for similar surgery and, of these, 22 had a dose-dependent increase in excretion on the first 2 days after operation. There were large individual variations. Exposure to 70% nitrous oxide appeared to cause abnormal metabolism of folate when exposure was greater than 90 min. Ten anaesthetists demonstrated normal excretion of formiminoglutamic acid; their exposure to nitrous oxide was typical of that in other studies of theatre environmental pollution.

KEY WORDS

Humans are exposed to nitrous oxide either acutely in high concentrations during general anaesthesia, or chronically in trace amounts from occupational exposure. It is postulated that, in some patients after surgery, this acute exposure may inhibit DNA synthesis, with consequent bone marrow depression and impaired resistance to infection. Investigations into this possibility have assessed the ability of exposed patients to synthesize new DNA, using the deoxyuridine suppression test [1] and direct assaying of liver methionine synthase activity [2]. Both methods are invasive, requiring bone marrow aspiration or liver biopsy.

Nitrous oxide inhibits methionine synthase [2, 3] by oxidation of the cobalt atom [4] in its cofactor, vitamin $B_{12}$. Methionine synthase plays a pivotal role in folate metabolism; it is the only method of regenerating tetrahydrofolate (THF) from methyl-THF [5]. Its inhibition causes an alteration in the composition of the folate pool, with both an increase in methyl-THF and a decrease in THF [6, 7]. If severe, this alteration interferes with DNA synthesis [8] because of decreased synthesis of deoxythymidine, one of the base precursors of DNA.

The functional state of the folate metabolic pathway may be assessed by examination of the urinary excretion of formiminoglutamic acid (FIGlu) [9–12]. Histidine is catabolized to FIGlu, which requires THF for further catabolism to glutamic acid. THF accepts the formimino group to form 5N-formimino-THF. When a deficiency of THF occurs, FIGlu cannot be metabolized fully and it accumulates in the plasma. Being water soluble, it is excreted in the urine. Small amounts are excreted normally and an increase in excretion occurs with THF deficiency. To expose minor abnormalities and to obviate the effects of diet [13], the histidine catabolic pathway may be stressed by ingestion of oral histidine [12]. This

P. ARMSTRONG, B.SC.(HONS), M.B., CH.B., F.C.ANAES., A. A. SPENCE, M.D., F.C.ANAES., F.R.C.P.(GLAS.); (Department of Anaesthesia); P. W. H. RAE, M.B., CH.B., M.R.C.P., PH.D.; (Department of Clinical Chemistry); University of Edinburgh, Royal Infirmary of Edinburgh, Lauriston Place, Edinburgh EH3 9YW. W. M. GRAY, B.SC., PH.D., West of Scotland Health Boards, Department of Clinical Physics and Bio-Engineering, 11 West Graham St, Glasgow G4 9LF. Accepted for Publication: September 27, 1990.
test gives a quantitative assessment of the functional state of folate metabolism [14-16].

We have used this relatively non-invasive FIGlu test to investigate the possibility that exposure to nitrous oxide, either during general anaesthesia or occupationally by anaesthetists, may cause abnormality in folate metabolism.

SUBJECTS AND METHODS

Approval for the study was granted by the local Ethics Committee and full informed consent obtained from all subjects. Urinary excretion of FIGlu was measured in all subjects in four groups. The control group consisted of 10 healthy subjects, not exposed to nitrous oxide. The surgical groups consisted of 55 patients undergoing limb surgery of at least 60 min duration: 50 (nitrous oxide group) received general anaesthesia including nitrous oxide; five (local anaesthetic group) received local anaesthesia. None had pre-existing renal or hepatic disease. The fourth group (anaesthetists) was composed of 10 anaesthetists who had been working full-time for at least 6 months. All subjects had full biochemical and haematological screening, including urea, creatinine, electrolytes, liver function tests, calcium, albumin, haemoglobin, red blood cell indices, white blood count, vitamin B12 and plasma and red blood cell folic acid concentrations. None was taking any drug known to interfere with folate metabolism.

Sample collection. The 10 control subjects were examined over 5 consecutive days. The 55 patients undergoing surgery were examined for 6 consecutive days, starting on the day before operation and continuing from the operative day to the 4th day after operation. The anaesthetists were examined daily for 7 consecutive days, starting at the beginning of the week. All anaesthetists carried out their normal daily work load.

Histidine loading. L-Histidine monochloride monohydrate 10 g was dissolved in 50 ml of either water or orange juice. Full dissolution in cold liquid was slow. The solution was drunk immediately, in the morning by the patients and in either the morning or the evening by the control subjects and anaesthetists.

Urine collection. Before ingestion of histidine, the bladder was emptied and all urine passed during the next 8 h [16] was collected in a plastic container, the bladder being emptied at the earliest convenient time subsequently. Concentrated hydrochloric acid 5 ml was added to each container to prevent decomposition of FIGlu [10]. The volume of each urine specimen was measured.

FIGlu estimation. FIGlu was measured by an established enzymatic method [17]. Standard solutions of urine samples were incubated at room temperature with the enzymes FIGlu transferase and formimino-THF cyclodeaminase (FIGlu enzymes, Sigma Chemical Company) in the presence of THF in the dark. The product of these enzymatic reactions is N6, N10-methenyl-THF, which readily undergoes hydrolysis to N10-formyl-THF. The addition of 10% perchloric acid at the end of the enzyme incubation converted any hydrolysed product back to N6, N10-methenyl-THF, which has an absorbance peak at 350 nm. Blanks were performed in parallel with all standards and the samples, differing only in that no enzyme was added. The absorbance at 350 nm of the blanks was subtracted from that of the appropriate standard or sample, and the concentrations in the samples estimated from a standard curve. The linearity of the assay extended beyond the working range required, and the absorbance of a standard solution of FIGlu agreed well with the calculated target value. The within-assay and between-assay coefficients of variation were 8% and 10%, respectively.

Anaesthetic. Both general and local anaesthetic techniques comprised oral temazepam 20 mg as a premedication 1 h before induction of anaesthesia with approximately 4 mg kg⁻¹ of thiopentone; suxamethonium was given to aid tracheal intubation. Maintenance of anaesthesia was with 70% nitrous oxide in oxygen; enflurane was given in addition as required. The duration of inhalation of nitrous oxide was noted. Nitrous oxide was given within 6 h of the histidine load. Papaveretum 10-20 mg was given i.v. and also used i.m. in the same dose for relief of postoperative pain. Local anaesthesia comprised either spinal or extradural injection of 0.5% heavy bupivacaine and 0.5% bupivacaine, respectively.

Exposure to nitrous oxide. Each anaesthetist's daily exposure to nitrous oxide was measured using passive diffusive samplers attached to the
NITROUS OXIDE AND FIGlu

TABLE I. Subject data for control and patient groups (mean (SD)). * Significantly different from the other groups (P < 0.001 Student's unpaired t test)

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>Sex (M:F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>30.1 (7.5)*</td>
<td>71.5 (9.7)</td>
<td>7:3</td>
</tr>
<tr>
<td>Nitrous oxide (n = 50)</td>
<td>51.7 (11.8)</td>
<td>69.0 (12.7)</td>
<td>24:26</td>
</tr>
<tr>
<td>Local anaesthesia (n = 5)</td>
<td>56.2 (5.1)</td>
<td>73.6 (9.7)</td>
<td>2:3</td>
</tr>
</tbody>
</table>

Fig. 1. Daily excretion of FIGlu in 10 control subjects not exposed to nitrous oxide.

theatre tunic near the breathing zone. These provided a time-weighted average exposure over the duration of the operating session [18].

Statistics. Patient and control data were analysed using Student's unpaired t test, as was FIGlu excretion between groups. FIGlu excretion within groups was analysed with ANOVA. Comparison of the mean total daily excretion of FIGlu by each anaesthetist for each of the 7 days and comparison of each anaesthetist's mean total excretion with that of the others was made with ANOVA.

RESULTS

Control subjects

The age of the control group was significantly less than that of the two patient groups (Student's unpaired t test, P < 0.001) (table 1); however, the age dependence of excretion of FIGlu is small [19], therefore this difference is unlikely to have invalidated comparisons between groups.

All 10 control subjects not exposed to nitrous oxide had normal biochemical and haematological data. All excreted some FIGlu each day (fig. 1), with an overall mean excretion of 27 (SD 22.0) μmol. The range of excretion was wide: 0.3–74 μmol. The mean total excretion of FIGlu for all the 10 controls was similar on each of the 5 days (ANOVA); no cumulative effects occurred. The total mean excretion of each individual control was similar (ANOVA).

Surgical groups

The operations performed on the groups of patients receiving local and general anaesthesia were similar. The local anaesthetic group comprised three total hip and two knee replacements; the nitrous oxide group comprised 17 total hip, 11 elbow and 15 knee replacements, five ankle and foot operations and two removal of infected plates. The duration of surgery for the two groups was similar: mean 111.4 (SD 33.1) min in the local anaesthetic group and 132.3 (56.5) min in the general anaesthetic group.

Local anaesthesia group. All patients receiving local anaesthesia had normal biochemical and haematological data. These subjects excreted FIGlu (fig. 2), on each of the 6 days tested, in amounts within the range previously observed in control subjects. There was no statistical increase in excretion on any of the study days (ANOVA).

Nitrous oxide anaesthesia group. In this group, the duration of anaesthesia was 64–312 min. Preoperative screening was normal in all except one individual who had a low concentration of vitamin B₁₂ (194 μmol; normal values are > 220 μmol). On the day before operation and the day of operation (days 1 and 2), all patients except one different individual on each day excreted FIGlu in amounts within the control range (fig. 3) (mean excretion on these days 24.8 (SD 22.3) μmol and 28.9 (23.6) μmol, respectively). However, an increase in excretion of FIGlu occurred on the first day after operation (day 3), when 22 patients excreted concentrations greater than those of the control group (> 80 μmol) (t test: P < 0.0001). There was a wide spread of values for FIGlu excreted, with a mean of 78.21 (67.38) μmol. A similar increase in excretion occurred on the next day (day 4), 12 of the 22 individuals excreting more than 80 μmol (t test: P < 0.0001 compared with controls). Mean excretion was 61.52 (50.98) μmol.

The increased excretion of FIGlu was dependent partially upon duration of exposure to nitrous oxide; those with increased excretions were exposed to the gas longer (mean 158.6...
FIG. 2. Daily excretion of FIGlu in five surgical patients receiving a local anaesthetic.

FIG. 3. Daily excretion of FIGlu in 50 surgical patients receiving nitrous oxide anaesthesia.

FIG. 4. Daily excretion of FIGlu in 10 anaesthetists occupationally exposed to nitrous oxide.

(64.4) min) than those with normal excretions (mean 113.3 (44.7) min) (Student’s unpaired t test: P < 0.008). Subjects exposed to nitrous oxide for less than 90 min had normal excretions and all those exposed for more than 211 min had increased excretions.

Excretion of FIGlu returned to control values on the next two days (days 5 and 6), with three and one (different) subjects excreting greater than normal amounts (means 25.49 (21.11) μmol and 29.80 (18.92) μmol, respectively). The patient with a low concentration of vitamin B₁₂ had normal FIGlu excretions throughout. The 22 subjects with excretion of FIGlu greater than 80 μmol on day 3 had plasma and red blood cell folate concentrations similar to those with lesser excretions.

Anaesthetists

The mean exposure of each anaesthetist to nitrous oxide was 53.4–159.2 p.p.m. (table II). The mean daily excretion of FIGlu of all the anaesthetists for each of the 7 days (fig. 4) was similar to that of the control subjects (Student’s unpaired t test). There was no significant difference in excretion between individuals or between mean total excretions on different days (ANOVA).

DISCUSSION

This study has demonstrated that exposure of patients to high concentrations of nitrous oxide during anaesthesia for limb surgery resulted in an increase in excretion of FIGlu on the first two days after operation in some individuals whilst anaesthetists, exposed to trace amounts, had no increase in excretion. Increased excretion was related to the duration of exposure to nitrous oxide and thus appeared to be dose-dependent.

Various workers are exposed chronically to nitrous oxide, including theatre personnel. Pollution of the operating room has caused much health concern [20], although there is little firm
The effect of nitrous oxide is thought to be both time- and concentration-dependent. Rats exposed to the gas continuously throughout pregnancy had increased fetal teratogenicity and resorption at a concentration of 1000 p.p.m., but not at 500 p.p.m. [22]. Intermittent exposures for 6 h per day, 5 days per week increased the threshold to between 1000 and 5000 p.p.m. [23]. Liver methionine synthase activity in rats was found to be unaffected by continuous exposure to concentrations of nitrous oxide less than 450 p.p.m., with an \( E_{50} \) of 5400 p.p.m. [24]. However, extrapolation of these animal studies to man is difficult. Human methionine synthase appears more resistant to nitrous oxide, with an inhibitory \( T_1 \) of 46 min, compared with 5.4 min for rats [2]. Moreover, the clinical effects of inhibition of methionine synthase differ between animals and humans [25]. Assessment of the possible dangers of nitrous oxide pollution has relied, therefore, on epidemiological studies of theatre workers, for whom liver biopsy for estimation of methionine synthase activity, and bone marrow sampling for marrow depression testing have obvious drawbacks. Three of 20 dentists exposed to nitrous oxide showed abnormal tests, although their exposure doses were greater than those found normally in theatre [26]. Other work investigating operating theatre workers by examination of peripheral blood films [27] and methionine concentrations [28] obtained negative findings.

Previous studies in patients undergoing nitrous oxide anaesthesia also have involved invasive measurement of methionine synthase activity [2, 29, 30] and investigations of the megaloblastic state of the bone marrow using the deoxyuridine suppression test or by direct morphological studies [31–36]. Therefore, there are few studies on the time-course of the effects of nitrous oxide.

Measurement of urinary excretion of FIGlu is a relatively non-invasive procedure, the only unpleasant aspect being the swallowing of the histidine solution. As this technique determines abnormalities in the folate metabolic pathway, it examines different aspects of nitrous oxide toxicity than those based on other methods. It is possible that partial inhibition of methionine synthase assessed in liver biopsies has no clinical significance, whilst abnormalities in bone marrow may indicate that gross biochemical changes have already occurred. Minor changes suggestive of early abnormality may not be revealed using this assay. As the folate pathway links these two biochemical events, investigation here may demonstrate a sensitivity to nitrous oxide toxicity superior to that of examination of the bone marrow, whilst being more clinically relevant than methionine synthase activity. Doses of histidine 10–20 g allow the diagnosis of folic acid deficiency to be made when there is minimal or no megaloblastosis in the marrow [9, 37].

Many regimens for histidine loading have been assessed, with doses of 1–45 g and with urine collections over different time intervals [16]. It has been found that, with the ingestion of 15 g or more, there is a high incidence of nausea and vomiting; with our 10-g loading dose, nine potential subjects were excluded from the study because of nausea. However, histidine 10 g is at least five times the normal dietary intake of histidine [13]. Following a histidine load, most FIGlu is excreted in the urine within 8 h [16].

The FIGlu test has been used in both rats and humans to assess the toxic effects of nitrous oxide. Rats exposed to 50 % for 24 h had an increase in urinary excretion of FIGlu [38]. Two of six humans anaesthetized with nitrous oxide at hyperbaric pressures (total dose equivalent to 3.1–7.0 h of 70 % nitrous oxide) had increased excretion of FIGlu [39]. We have demonstrated a similar response. Exposure to nitrous oxide for clinical anaesthesia caused an increase in urinary excretion of FIGlu in approximately 50 % of subjects. No increase occurred on the day of operation, probably because exposure to nitrous oxide usually occurred long after the histidine load was given and most of the histidine would have been metabolized before the exposure. This is not a surgical effect, as no increase occurred in the local anaesthetic group and it is unlikely to have been caused by a drug other than nitrous oxide, as there has been no report of abnormal folate metabolism with other anaesthetics.

It is not clear why only some individuals are affected by nitrous oxide. Recent evidence implicates the hydroxyl radical scavenger dimethylthiourea as responsible for inactivation of methionine synthase; a hydroxyl radical scavenger protects the enzyme from inactivation by nitrous oxide [40]. It appears that nitrous oxide may react with the cobalt atom in vitamin \( B_{12} \) as follows:

\[
\text{Co}(I) + N_2O \rightarrow \text{Co}(II) + N_2 + \text{OH}'
\]

The hydroxyl radical (\( \text{OH}' \)) may then attack amino acids around the active site of methionine synthase.
Synthase, thereby inactivating it irreversibly [41]. Hydroxyl radicals are highly reactive and readily attack amino acids, especially those that contain sulphur [42]. The cytoplasmic concentrations of intrinsic hydroxyl radical scavengers differ between human individuals, and this may be responsible for different degrees of human susceptibility to nitrous oxide.

The return of FIGlu excretion to normal 72 h after exposure to nitrous oxide is consistent with the irreversibility of the inactivation of methionine synthase, with a requirement for synthesis of new enzyme [43, 44].

One patient had abnormally small concentrations of vitamin B\textsubscript{12} before exposure to nitrous oxide, although he had no increase in excretion of FIGlu. Nitrous oxide toxicity is increased in animals made deficient in vitamin B\textsubscript{12} [45]. The reason for the lack of effect in this patient is unknown.

This work demonstrates that exposure to increased concentrations of nitrous oxide for at least 90 min caused an abnormality in the folate metabolic pathway. The eventual consequence of this would be a decrease in DNA synthesis. In humans, exposure to 50\% nitrous oxide for at least 6 h is needed for this to be significant; in our study, patients received 70\% for up to 320 min and some demonstrated abnormalities in their folate metabolism. The clinical relevance of this result is unclear at present.

In contrast to individuals exposed acutely to increased concentrations of nitrous oxide, in anaesthetists who were exposed chronically to trace concentrations of nitrous oxide, the daily urine excretion of FIGlu was similar to that of the control group; this suggests that theatre pollution by nitrous oxide has no adverse effects on folate metabolism of exposed workers, which is in agreement with two other studies [9, 28].

The target maximum exposure to nitrous oxide set by the United States National Institute for Occupational Safety and Health (NIOSH) is 25 p.p.m. [46], a value which was almost always exceeded in this study. Sharer and others [24] have recommended a safe limit of 200 p.p.m. and it would appear from our study also that the NIOSH limit may be unnecessarily low.

ACKNOWLEDGEMENT
Dr Armstrong was in receipt of a research fellowship from the Association of Anaesthetists of Great Britain and Ireland.

REFERENCES
NITROUS OXIDE AND FIGlu