When Nitrous Oxide is No Laughing Matter

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First synthesized by Joseph Priestley in 1772, the psychotropic effects of nitrous oxide were first appreciated (in the first person) by Humphrey Davy. From the time that nitrous oxide was first used for analgesia by Horace Wells in December 1844 until 1956, nitrous oxide was considered to be completely benign. Although there were two isolated case reports, the first clear association of nitrous oxide and hematologic disease came in a report by Lassen, et al. in the Lancet in 1956.(1) They reported several patients with tetanus who were sedated with nitrous oxide for several days and developed evidence of aplastic anemia. They showed that the only drug that completely differentiated these patients from their patients who did not develop hematologic problems was nitrous oxide. They then studied it prospectively in a 10 year old boy. Granulocytopenia developed on the fourth day (of 50% N\textsubscript{2}O), the N\textsubscript{2}O was discontinued and thrombocytopenia followed within several days. A bone marrow biopsy was consistent with pernicious anemia with megaloblastic changes. The hematologic picture resolved after discontinuation of N\textsubscript{2}O. A similar picture was reported in cardiac surgery patients kept on 50% N\textsubscript{2}O for 24 hours during and after cardiac surgery.(2) A six hour exposure resulted in mild megaloblastic changes, and a 24 hour exposure in severe changes.

In 1978, Sahenk reported a case of polyneuropathy from recreational N\textsubscript{2}O use(3) and Layzer reported on two dentists and a hospital technician who used N\textsubscript{2}O recreationally several times a week and developed a sensory polyneuropathy which resolved on discontinuation of N\textsubscript{2}O.(4) Layzer in a follow-up paper reported on 15 patients, 14 of whom were dentists.(5) The polyneuropathy was likened to that of vitamin B\textsubscript{12} deficiency. Several dentists were not recreational users but had heavy exposure in the course of their work. The concentration of inhaled N\textsubscript{2}O varied between 30-80%, and in some was never higher than 50%. The course of recovery was unaffected by treatment with steroids or vitamin B\textsubscript{12}. In a survey of 18,000 dentists and 18,000 dental assistants, Jay Brodsky was able to show that there was a gender independent association of neurologic findings similar to those of pernicious anemia with nitrous exposure.(6)

Nitrous oxide irreversibly oxidizes the cobalt atom of vitamin B\textsubscript{12}, transferring it from the active Co(I) state to the inactive Co(II or III) state, inactivating it, somewhat analogous to the reduction of hemoglobin to methemoglobin. In this reaction nitrous oxide is reduced to nitrogen and oxygen. Although originally described by Banks in 1968,(7) this finding was not appreciated in the medical community. This eventually led to an investigation of the effects of N\textsubscript{2}O on a variety of B\textsubscript{12}-dependent enzyme systems, particularly by John Nunn and his group.

Methionine is an essential amino acid that serves as a methyl donor (via its activated form S-adenosylmethionine) in hundreds of biologic reactions. The end product of methionine demethylation is homocysteine, whose remethylation is catalyzed by the vitamin B\textsubscript{12} dependent enzyme methionine synthase (synthetase).
Methionine synthetase is a cytosolic enzyme. It catalyzes the conversion of homocysteine + methyltetrahydrofolate to methionine + tetrahydrofolate. The methylcobalamin form of vitamin B$_{12}$ is a cofactor. Inhibition of this enzyme by N$_2$O was first shown in rats, where 50% N$_2$O caused a fall in enzyme activity within 30 minutes and activity was essentially absent after six hours.(8) In mice, 50% inhibition of enzyme activity occurs after a four hour exposure to 0.1 atm, with recovery complete by 2-4 days after a four hour exposure to 0.8 atm..(9) However, the onset of inhibition appears to be slower in man than in small rodents. In a recent abstract Culley and her coworkers showed that a four hour exposure to 70% N$_2$O in rats produced lasting memory impairment which is preceded by a reduction in cerebral cortical methionine synthase activity.(10)

Homocystinuria

Homocystinuria is the second most common disease of amino acid metabolism. An autosomal recessive disease, it is due to abnormalities in one of three genes involved in the catabolism of methionine and the conversion of homocysteine to methionine. The most common type, type I, accounting for 95% of cases, is due to a defect in cystathionine synthetase, which catalyzes the synthesis of cystathionine from homocysteine and serine. This type utilizes vitamin B$_6$ as a cofactor, and is unaffected by N$_2$O. There are two subtypes, one of which is B$_6$ responsive, and one B$_6$ unresponsive. Type II disease is caused by a defect in the enzyme tetrahydrofolate methyltransferase, and type III by a defect in 5,10-methylenetetrahydrofolate reductase (MTHFR).

These eventually result in a defect in the transsulfuration of the precursors of cysteine, which then results in weakened cross-linking of collagen. These patients have a Marfanoid habitus with lens dislocation, pectus excavatum, premature coronary artery disease, arterial and venous thromboembolic disease, strokes, osteoporosis, and scoliosis being commonly encountered. There can also be hyperinsulinism and hypoglycemia, from exposure of the pancreas to sulfur-containing amino acids. Unlike Marfan syndrome, joints are not hyperflexible and there is an incidence of mental retardation, a major finding in type III, presumably due to a deficiency in methionine, an essential amino acid. Methionine, by way of its activated form, S-adenosylmethionine, is a methyl donor in many reactions, including myelin sheath assembly, neurotransmitter synthesis and DNA synthesis in rapidly proliferating tissues. Spontaneous thromboemboli are presumably a sequela of fraying of collagen in the vessel wall with loss of overlying endothelium, or activation of Hageman factor by homocysteine. Perioperative concerns include coronary artery disease risk, possible hypoglycemia, and thromboembolic risk which is heparin unresponsive. Dextran infusions have been suggested to minimize the hypercoagulable state. There are no reports on specific inhibitors of platelet function.

Nitrous oxide inhibits the activity of methionine synthetase, which converts homocysteine to methionine, raising homocysteine levels. It has been suggested that N$_2$O be avoided in these patients. There has been a report of a fatal outcome in a patient with type III disease due to compound heterozygosity including a novel MTHFR mutation (1755 G→A), which was inherited in concert with two common MTHFR polymorphisms, both of which are associated with diminished enzyme activity.(11) This previously undiagnosed child had exposure to N$_2$O twice within four days. Twenty-five days after the first exposure seizures and apnea developed, with hypotonia and areflexia.
Death was from a respiratory arrest on postoperative day 46. Presumably the N\textsubscript{2}O induced inhibition of methionine synthetase (see below) in addition to the genetic defect in tetrahydrofolate reductase and led to death secondary to methionine deficiency.

Although as pediatric anesthesiologists one typically thinks of MTHFR and its relationship to homocystinuria, homocystinuria is an uncommon disease, but specific mutations in this gene (single nucleotide polymorphisms, SNPs) are common. One SNP (C>T\textsuperscript{677}), for example, is associated with mild homocystinemia, but not deep vein thrombosis. This missense mutation in MTHFR has been associated with preeclampsia.(12) Heterozygosity for this SNP occurs in 12-57% of the population. Lacassie et al. have reported a patient who received N\textsubscript{2}O twice within a period of 10 weeks who subsequently developed myelopathy and a macrocytic anemia. Further evaluation showed elevated homocysteine levels, low B\textsubscript{12} levels, and the C>T\textsuperscript{677} SNP in MTHFR.(13) Neurologic findings resolved with supplementation with folate and B\textsubscript{12}. Although screening of all patients for elevations in homocysteine plasma levels prior to anesthesia is impractical, with the association of elevated homocysteine levels with coronary disease, more people are having this done. If found, measures of methionine levels might also be considered.(14) If found, fortuitously or otherwise, it must be remembered that close family members may also share this SNP.

**Methylmalonic Acidemia**

This disorder is due to a defect in one of several enzymes that result in the accumulation of methylmalonic acid. These include methylmalonyl-coenzyme A mutase, a defect in adenosylcobalamin synthesis causing impaired mutase function, or a defect in both adenosylcobalamin synthesis (B\textsubscript{12}-dependent) and the methylcobalamin-dependent enzyme N5-methylenetetrahydrofolate methyltransferase (which results in methylmalonic aciduria and homocystinuria). This disease presents like many of the aminoacidopathies with episodes of severe metabolic acidosis, ketosis and hyperammonemia at times of increased protein catabolism. They can also have growth retardation, osteoporosis, hypoglycemia and recurrent vomiting.

Rask et al. showed that 24 hours of nitrous oxide can increase urinary methylmalonic acid levels threefold in normal patients.(15) Sharar et al. reported on the anesthetic management of a child with methylmalonic acidemia from methylmalonyl-coenzyme A mutase deficiency.(16) This mitochondrial enzyme normally converts methylmalonyl-Co (formed from the degradation of a variety of branched chain amino acids, cholesterol and some fatty acids) to succinyl-CoA, with B\textsubscript{12} (adenosylcobalamin) as a cofactor. With insufficient activity of this enzyme, its substrate, methylmalonyl CoA, is converted to methylmalonic acid. They commented on the theoretical risk of nitrous oxide on this B\textsubscript{12}-dependent enzyme, and it was avoided. Interestingly, Deacon had shown earlier that this B\textsubscript{12}-dependent enzyme was unaffected by N\textsubscript{2}O, at least in rats.(8), so the true clinical implications in this disorder are unclear, although avoidance of nitrous oxide would seem prudent.

**Other B\textsubscript{12} Related Effects**

A variety of situations can result in B\textsubscript{12} deficiency. There have been a handful of case reports of patients with B\textsubscript{12} deficiency from resection of the terminal ileum or from
pernicious anemia who have developed neurologic manifestations of B₁₂ deficiency after intraoperative exposure to N₂O. The duration of anesthesia in these cases was 90-235 minutes and the onset of symptoms was from 14 days to eight weeks after surgery. B₁₂ deficiency induced by imposed dietary restrictions can also occur. There have been two cases of neurologic and hematologic sequelae in infants with dietary B₁₂ deficiency. Felmet et al. reported an eight month old who developed bone marrow failure and severe neuropathy following an 80 minute exposure to N₂O (concentration unknown). (17) He had been solely breastfed by a mother who was B₁₂ deficient from deficiency of intrinsic factor. McNeely et al. described a six-month old girl with B₁₂ deficiency of undetermined etiology (although her mother was an “almost” vegan and the infant was breastfed) who had surgery for three hours with N₂O (concentration not specified). She developed hypotonia and anemia beginning three weeks postoperatively. (18) In an adult, Rosener and Dichgans presented the case of dietary B₁₂ deficiency in a 50 year who had been a vegetarian for 10 years, and whose diet was restricted to apples, nuts and raw vegetables, with no legumes. At the time of surgery she had a mild macrocytic anemia. She required surgery for a fractured hip which involved 66% N₂O for two hours. Four weeks later she developed an unsteady gait and decreased sensation in the legs. By six weeks postoperatively she was unable to walk and her macrocytic anemia had worsened. She improved with injections of B₁₂ but without total return of function. (19)

B₁₂ deficiency has also been reported in adolescents with phenylketonuria. These people are placed on protein restricted diets, which will also limit intake of B₁₂. (20)

Non-B₁₂ Mediated Effects

Nitrous oxide has been shown to have neurotoxic effects in both developing and adult brains in in vivo studies in rats. Interestingly, the pathophysiology differs. In developing brain, the mechanism appears to be one of inducing apoptotic death. However, N₂O alone has no effect. Rather, it potentiates the apoptotic neurodegeneration induced by even low dose (0.75%) isoflurane. (21) Exposure to nitrous oxide alone, however, is neurotoxic to both adult and aging brain. (22) In these animals the neuropathic changes are vacuolization with swelling of the mitochondria and endoplasmic reticulum and not apoptosis. Interestingly, apoptosis is a naturally occurring phenomenon in rapidly developing immature brain, but not adult brain. It has been suggested that the effect in adult brain is due to nitrous oxide’s effect as an NMDA receptor antagonist. In adult rats, three hours of N₂O exposure resulted in transient vacuolization, but eight hours exposure resulted in neuronal cell death. (23) Culley in a recent abstract showed somewhat impaired memory in the equivalent of late middle aged (18 month old) rats that received four hours of 70% N₂O. (10) This was preceded by reduced activity of cortical methionine synthase. However, although a temporal relationship could be shown, there was no documentation of a causal relationship.
References


Questions

1. Toxicity of nitrous oxide might be manifest in which organ systems?
   A. Liver
   B. Hematopoietic
   C. CNS
   D. Renal
   E. Thyroid

2. Nitrous oxide inhibits a vitamin [and is it permanent inhibition, and why (or why not)]?
   A. D
   B. B\textsubscript{12}
   C. Thiamine
   D. E
   E. Niacin

3. What clinical features distinguish homocystinuria from Marfan syndrome?
   A. Optic lens dislocation
   B. Mental retardation
   C. Tall stature
   D. Pectus excavatum
   E. Hyperextensible joints

4. 5,10-methylenetetrahydrofolate reductase (MTHFR) single nucleotide polymorphisms are not uncommon. Why should we care?

5. What difference does it matter which type of homocystinuria a patient has?