When nitrous oxide is no laughing matter: nitrous oxide and pediatric anesthesia

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Summary
Although often felt to be relatively innocuous, nitrous oxide can have significant metabolic effects in settings of abnormal vitamin B12 and B12-related metabolism in children. These conditions can be genetic or environmental. Symptoms may not appear until days to weeks after exposure to nitrous oxide. Although overt genetic diseases are relatively uncommon, the implications of nitrous oxide interactions with much more frequent but less symptomatically obvious single nucleotide polymorphisms are potentially more concerning. In addition, nitrous oxide can have direct and differing neurotoxic effects on both immature and aged brain, the clinical impact of which remains undetermined.

Keywords: 5,10-methylenetetrahydrofolate reductase; homocystinuria; methionine synthetase; methylmalonic acidemia; nitrous oxide; vitamin B12

Although widely used for many decades, there are certain clinical situations where nitrous oxide should be avoided. In particular, there are several genetic metabolic conditions where the use of nitrous oxide can have fatal consequences. Although some reference to adult humans and experimental animals is unavoidable, this review will specifically address pediatric conditions, and will concern itself primarily with biochemical interactions and limitations, rather than physical issues such as expansion of closed air spaces, hemodynamic effects or pollution concerns. Finally, in 2007, no discussion of nitrous oxide and children is possible without at least passing note of the somewhat contentious effects of nitrous oxide on the immature brain.

Several recent reviews of the effects of nitrous oxide have reached widely differing conclusions regarding the appropriate role of this drug in modern practice (1–3) from positive (3) to 'Nitrous oxide – an outdated anaesthetic' (2), and there is currently a large clinical trial in adults looking at clinical outcomes with the use of nitrous oxide (4). These reviews do not specifically address issues in pediatric anesthesia and address minimally or not at all the effects of nitrous oxide in genetic diseases as will be discussed below.

First synthesized by Joseph Priestley in 1772, the psychotropic effects of nitrous oxide were first appreciated (in the first person) by Humphrey Davy in 1799 (5), who encouraged its recreational use by his friends including Coleridge and Roget. Although Davy’s recommendation that it be used as anesthetic for minor surgery was not heeded, his
experience began the fad of recreational use at parties. From that time through the first use of nitrous oxide for analgesia by Horace Wells in December 1844, and until 1956, nitrous oxide was considered to be completely benign. In fact, an article in the lay publication *Popular Science* in 1949 gave directions for the synthesis of nitrous oxide in a simple home laboratory (Fig. 1). Although there had been two prior 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 (6). 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% N₂O), the N₂O 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 N₂O. A similar picture was reported in cardiac surgery patients kept on 50% N₂O for 24 h during and after cardiac surgery (7). A 6 h exposure resulted in mild megaloblastic changes, and a 24 h exposure in severe changes.

In 1978, Sahenk et al. reported a case of polyneuropathy from recreational N₂O use (8) and Layzer et al. reported on two dentists and a hospital technician who used N₂O recreationally several times a week and developed a sensory polyneuropathy that resolved on discontinuation of N₂O (9). Layzer in a follow-up paper reported on 15 patients, 14 of whom were dentists (10). The polyneuropathy was likened to that of vitamin B₁₂ deficiency. Several of these dentists were not recreational users but had heavy occupational exposure. The concentration of inhaled N₂O varied between 30% and 80%, and in some was never higher than 50%. The course of recovery was unaffected by treatment with steroids or vitamin B₁₂. In a survey of 18 000 dentists and 18 000 dental assistants, Brodsky et al. were able to show that with nitrous exposure there was a gender independent association of neurologic findings similar to those of pernicious anemia (11).

Nitrous oxide irreversibly oxidizes the cobalt atom of vitamin B₁₂, transferring it from the active Co(I) state to the inactive Co(II or III) state, inactive it, somewhat analogous to the reduction of iron, converting hemoglobin to methemoglobin. In this reaction, nitrous oxide is reduced to nitrogen and oxygen. Although originally described by Banks et al. in 1968 in the chemistry literature (12), this finding was not appreciated immediately in the medical community. Eventually, this information led to an investigation of the effects of N₂O on a variety of B₁₂-dependent enzyme systems, particularly by Nunn and his group.

**Metabolic diseases**

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 B₁₂ dependent enzyme methionine synthase (synthetase). Inhibition of methionine metabolism by inactivation of B₁₂ is a major locus for the metabolic effects of N₂O (Fig. 2).

**Methionine synthetase**

Methionine synthetase is a cytosolic enzyme catalyzing the conversion of homocysteine + methyltetrahydrofolate to methionine + tetrahydrofolate. The methylcobalamin form of vitamin B₁₂ is a cofactor. Deficiency in this enzyme manifests with megaloblastic changes, growth retardation,
psychomotor retardation, neurologic problems and elevated levels of homocysteine and diminished levels of methionine.

Inhibition of this enzyme by N₂O was first shown in rats, where 50% N₂O caused a fall in enzyme activity within 30 min and activity was essentially absent after 6 h (13). In mice, 50% inhibition of enzyme activity occurs after a 4 h exposure to 0.1 atm, and recovery is complete by 2–4 days after a 4 h exposure to 0.8 atm (14). In a recent abstract Culley and her coworkers showed that a 4 h exposure to 70% N₂O in the equivalent of late middle aged (18 month old) rats produced lasting memory impairment which was preceded by a reduction in cerebral cortical methionine synthase activity (15). Although implied in this brief report, this study did not attempt to confirm a direct causal relationship of impaired methionine synthetase activity and memory impairment despite the temporal relationship. The onset of inhibition appears, however, to be slower in man than in small rodents. Enzyme activity in patients with methionine synthetase deficiency is already lacking and presumably is not made worse by N₂O. However, the result of superimposition of inhibition of B₁₂ mediated related metabolic pathways is not known and the implications of a brief exposure of clinically relevant levels of N₂O in patients with methionine synthetase deficiency remain unknown. It would seem reasonable, however, to avoid it in patients with this condition.

**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 (Fig. 2). 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 B₆ as a cofactor, and is unaffected by N₂O. There are two subtypes, one of which is B₆ responsive and one B₆ 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 enzyme defects 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.

There has been a report of a fatal outcome in a 3-month-old boy with type III disease due to compound heterozygosity including a novel MTHFR mutation (G > A³⁷⁵⁵), which was inherited in concert with two common MTHFR polymorphisms, both of which are associated with diminished enzyme activity (16). This previously undiagnosed child had exposure to N₂O twice within 4 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 N₂O induced inhibition of methionine synthetase (see above) 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.
Specific mutations in this gene (single nucleotide polymorphisms, SNPs) are, however, common. One SNP (C > T677), for example, is associated with mild homocystinemia, but not deep vein thrombosis. This missense mutation in MTHFR has been associated with preeclampsia (17). Heterozygosity for this SNP occurs in 12–57% of the population. Lacassie et al. have reported a patient who received N₂O twice within a period of 10 weeks who subsequently developed myelopathy and a macrocytic anemia. Further evaluation showed elevated homocysteine levels, low B₁₂ levels, and the C > T⁶⁷⁷ SNP in MTHFR (18). Neurologic findings resolved with folate and B₁₂ supplementation.

Because of the association of homocystinuria and premature coronary artery disease, elevated plasma homocysteine has been identified as a risk factor in adults for coronary artery disease (19), and has been associated with worse prognosis (20). Because of this, it is possible that some adult patients will have had their plasma homocysteine levels measured, although screening of all patients for elevations in homocysteine plasma levels before anesthesia is impractical. If found, measures of plasma methionine levels might also be considered (21). If elevated homocysteine levels in concert with depressed methionine levels are found, fortuitously or otherwise, it must be remembered that close family members, including children, may also share this SNP and be at risk with N₂O exposure.

**Methylmalonic acidemia**

This disorder is 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 (B₁₂ 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. Affected individuals can also have growth retardation, osteoporosis, hypoglycemia and recurrent vomiting. Rask et al. showed that 24 h of nitrous oxide can increase urinary methylmalonic acid levels threefold in normal patients (22). Sharar et al. reported on the anesthetic management of an 11-year-old girl with methylmalonic acidemia from methylmalonyl-coenzyme A mutase deficiency (23). 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 B₁₂ (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 B₁₂-dependent enzyme, and it was avoided. Interestingly, Deacon had shown earlier that this B₁₂-dependent enzyme was unaffected by N₂O, at least in rats (13), so although the true clinical implications in this disorder are unclear, avoidance of nitrous oxide would seem prudent.

**Other B₁₂ related effects**

A variety of situations can result in B₁₂ deficiency. There have been a handful of case reports of patients with B₁₂ 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 min and the onset of symptoms was from 14 days to 8 weeks after surgery. B₁₂ deficiency induced by imposed dietary restrictions can also occur. There have been two known cases of neurologic and hematologic sequelae in infants with dietary B₁₂ deficiency. Felmet et al. reported an 8 month old who developed bone marrow failure and severe neuropathy following an 80 min exposure to N₂O (concentration unknown) (24). He had been solely breastfed by a mother who was B₁₂ deficient from deficiency of intrinsic factor. McNeely et al. described a 6-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 3 h with N₂O (concentration not specified). She developed hypotonia and anemia beginning 3 weeks postoperatively (25). 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

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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 2 h. Four weeks later, she developed an unsteady gait and decreased sensation in the legs. By 6 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 (26).

B₁₂ deficiency has also been reported in adolescents with phenylketonuria. These individuals are placed on protein-restricted diets, which will also limit intake of B₁₂ (27). Although there is no other published literature, one must wonder about other aminoacidopathies treated with protein-restricted diets.

Non-B₁₂ mediated effects

Over the past several years, there has been experimental work which has shown significant neural toxicity of N₂O. Because the findings are somewhat contentious and the clinical implications are as yet unknown but are potentially extremely important given the wide use of N₂O, this deserves at least some mention. A recent review of the effects of anesthetics on the immature brain primarily concerned itself with NMDA and GABA effects, primarily of ketamine, about which most is known (28). That review confirmed that there are currently no clinical data in humans to confirm implications of neural injury in laboratory animals.

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 between developing and adult brain. In developing brain, the mechanism appears to be one of induced apoptotic death. Jevtovic-Todorovic and coworkers have suggested that N₂O alone has no effect. Rather, it potentiates the apoptotic neurodegeneration induced by even low dose (0.75%) isoflurane (in immature rats) (29). Januszewski et al., however, in a recent study in immature mouse hippocampal slices have shown that both N₂O (75%) and isoflurane (0.75%) independently and in combination can significantly increase the expression of caspase-3, a marker of apoptosis (30).

Exposure to nitrous oxide alone, however, is neurotoxic to both adult and aging brain in experimental settings (31). In adult rats, 3 h of N₂O exposure resulted in transient vacuolization, but 8 h exposure resulted in neuronal cell death (32). 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, although I am unaware of studies investigating interactions of nitrous oxide and methionine metabolism in rapidly developing brain with high DNA turnover.

Summary

Nitrous oxide exerts many of its deleterious effects via an irreversible interaction with vitamin B₁₂. In the presence of B₁₂ deficiency profound effects of N₂O can be seen after even brief exposure to clinical levels of N₂O and the onset of clinical findings can be delayed for weeks. Although these effects are pronounced in the relatively small number of patients with metabolic disorders related to B₁₂ mediated processes, the overall effects on the much larger number of patients having single nucleotide polymorphisms of these genes is unclear. Nitrous oxide also has significant effects on neural cell death in both immature and aging brain as has been shown in experimental animal models. However, the implications for the human fetus and infant, where the brain is maturing at a slower rate than in rodents, remain a matter of ongoing controversy.

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