

# Pharmacogenetics of Nitrous Oxide

## Standing at the Crossroads

IT has long been known that nitrous oxide in clinically relevant doses and durations shuts down single carbon pathways by virtue of oxidation of the cobalamin cofactor of methionine synthase, the sole occupant at the crossroads of the methylation, transsulfuration, and folate cycles. Surprisingly, the clinical consequences of nitrous oxide-mediated, folate-cobalamin cycle inactivation have not restrained its use in the more than 150 yr that have elapsed since its introduction for surgical anesthesia. In part, this may be explained by preoperative cellular stores of *S*-adenosyl methionine that are adequate for most patients to ride out the days needed for enzyme resynthesis. In the current issue of ANESTHESIOLOGY, Nagele *et al.*<sup>1</sup> suggest a corollary explanation. In their contribution, the authors show that although all patients experience modest elevations in homocysteine (*i.e.*, *S*-adenosyl methionine's amino acid substrate) after exposure to nitrous oxide, patients who are homozygous for polymorphisms in the gene encoding 5,10 methylene tetrahydrofolate reductase (*MTHFR*), the enzyme antecedent to methionine synthase in the folate cycle, may develop levels well above those associated with adverse pathophysiologic consequences. If confirmed and extended, these observations suggest that all patients are not equally at risk for homocysteine-associated nitrous oxide toxicity, that deleterious events after nitrous oxide exposure may be enriched in a subset of patients at greatest genetic predisposition, and that risk stratification by genotype may be a useful adjunct in judging who should inhale nitrous oxide, at what concentration, and for how long.

Superimposition of an acquired lesion in methionine synthase on inborn lesions in 5,10 methylene tetrahydrofolate reductase leads to conjoined biochemical alterations, *i.e.*, accumulation of the reaction substrate homocysteine, and deficiency of the reaction product *S*-adenosyl methionine. *S*-Adenosyl methionine is the direct methyl donor in more than 100 metabolic steps,

including methylation of DNA, RNA, proteins, phospholipids, myelin, polysaccharides, choline, and neurotransmitters. Accordingly, hypomethylation impairs gene expression, cell growth, differentiation, and function that may underlie multiple conditions associated with prolonged or ill-timed exposure to nitrous oxide, including bone marrow depression, subacute combined degeneration of the spinal cord in adults after use and abuse, hazards of unscavenged occupational exposure, and neuronal toxicity in childhood.<sup>2</sup> The role, if any, of hypomethylation secondary to genetic predispositions in these nitrous oxide syndromes is provocative, although incompletely understood at present.<sup>3</sup> Homocysteine may accumulate as an innocent bystander, or as a non-specific marker of enzyme inactivation, rather than as a primary participant in the pathologic cascade of methyl deficiency.

In more common disorders of central interest to anesthesiologists, however, hyperhomocysteinemia rather than hypomethylation may be the dominant lesion. Moderate hyperhomocysteinemia (*i.e.*, 12–30  $\mu\text{M}$ ) most frequently arises from vitamin deficiency, renal insufficiency, and, as here, *MTHFR* mutations. Intermediate homocysteine levels between 30 and 100  $\mu\text{M}$  are often due to profound vitamin deficiency, kidney failure, and heterozygosity for cystathionine  $\beta$ -synthase (*CBS*) polymorphisms, whereas severe elevations ( $> 100 \mu\text{M}$ ) are seen in rare patients with homozygous *CBS* mutations.<sup>4</sup> Moderate to intermediate elevation of homocysteine injures the vascular endothelium, promotes coagulation, and impairs smooth muscle relaxation *via* inhibition of nitric oxide bioavailability, inactivation of factor  $\text{V}_a$ , reduced antithrombin II and tissue plasminogen activator binding, increased resistance to fibrinolysis, vascular smooth muscle and foam cell proliferation, enhanced platelet activation and aggregation, increased expression of proinflammatory cytokines, and formation of reactive oxygen species, among numerous other established and proposed mechanisms.<sup>5</sup> Whereas the association of hyperhomocysteinemia and venous thromboembolism is controversial on the basis of currently available clinical data, chronically elevated plasma homocysteinemia is recognized as an independent risk factor for atherosclerosis and related arterial occlusive conditions (*e.g.*, restenosis), particularly in patients with coexisting cardiovascular disease, acquired folate or cobalamin deficiencies, and *MTHFR* mutations.<sup>6</sup>

Hyperhomocysteinemia is also an independent risk factor for neurodegenerative diseases and dementia. In neurons, homocysteine competes for glutamate and aspartate receptors, and exacerbates oxidative stress, for-

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mation of hydroxyl radicals, intracellular calcium shifts, and apoptosis *via* excitotoxicity.<sup>7</sup> Moreover, homocysteine potentiates  $\beta$ -amyloid toxicity in cultured neurons, in transgenic mice, and in humans.<sup>8</sup> Moderately elevated plasma homocysteine levels correlate inversely with cognitive function and memory deficits, with a doubling of risk for developing Alzheimer disease in patients with total plasma homocysteine greater than  $14 \mu\text{M}$  compared with patients with normal levels.<sup>9,10</sup> While hyperhomocysteinemia can directly damage neurons, it may also contribute to dementia through disruption of cerebrovascular endothelium. Decreasing plasma homocysteine reduces the risk of stroke by 20%, a decrement greater than that observed after therapy for hyperhomocysteinemia in cardiovascular diseases.<sup>11</sup>

With the multitude of cellular disruptions associated with hyperhomocysteinemia in mind, several provisos are warranted in interpreting the data reported by Nagele *et al.* First, in the current investigation, only a single concentration and two durations of nitrous oxide exposure are described. Further elucidation of nitrous oxide concentrations and durations *versus* plasma homocysteine levels are required. Second, when the final sample was collected at the end of surgery, homocysteine was on its way up in patients with all genotypes. As the authors note, the detrimental effects of homocysteine probably extend well into the postoperative time frame. In the future, it will be essential to expand the sampling interval for however long it takes to capture peak levels, and to define the time course for return to baseline, both of which may be influenced by genotype. Third, Nagele *et al.* report the association of homocysteine levels after nitrous oxide with just two polymorphisms in a single gene. In view of the “double-hit” mechanism proposed by Selzer *et al.* and confirmed by Nagele *et al.*, at least a dozen additional polymorphisms in genes encoding enzymes that participate in single carbon cycles are compelling candidates for companion investigations.<sup>12</sup> Finally, myriad factors other than single-carbon genotypes and nitrous oxide influence homocysteine metabolism and contribute to total plasma homocysteine levels, including commonly used medications (*e.g.*, antibiotics, antiepileptics, L-dopa, isoniazid), intercurrent disorders (*e.g.*, proliferative disorders, hypothyroidism, peripheral vascular disease), and lifestyle choices (*e.g.*, exercise, diet, alcohol and coffee consumption).<sup>4,5</sup> To preclude spurious claims for genotype:phenotype correlations in teasing out nitrous oxide pharmacogenetics, it will be essential to take into account and, whenever possible, to control these variables.

Even a brief overview of the causes and consequences of hyperhomocysteinemia begs the question, should the data of Nagele *et al.* concern anesthesiologists contemplating nitrous oxide use? A significant constraint in

applying knowledge of hyperhomocysteinemia to the surgical setting is that many *in vitro* and tissue endpoints have been observed with higher levels of homocysteine than those proven to be reached in clinical practice. As well, a corresponding limitation in human investigations is that the preponderance of evidence addresses the effects of chronic rather than acute hyperhomocysteinemia, although exceptions exist.<sup>13</sup> Although Nagele *et al.* do not report clinically relevant outcomes in patients with and without acute nitrous oxide exposure, the authors provide a solid step forward for future investigations to follow. Clearly, trials of nitrous oxide exposure in any clinical setting, including surgery, pediatric sedation, the dental office, the emergency room, and the birthing suite, will be substantially strengthened by inclusion of single-carbon genotyping to assure that patients with shared genotypes are compared with one another. In turn, knowing in advance who can breathe nitrous oxide without fear of clinically relevant hyperhomocysteinemia may help to sustain its safe use for a second century and a half, and beyond.

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