Nitrous oxide in 2010: who will have the last laugh? (Part 1)

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Introduction

Nitrous oxide (N_2O) has been a part of anaesthetic practice for over 150 years. During this time, its reputation has seesawed. Today, anaesthetic opinion seems to have swung away from its use. To ascertain whether this is based on sound scientific principles, or can be ascribed to a shift in "medical fashion", a Pubmed search for all articles containing the terms "nitrous oxide" and "anaesthesia" was conducted for the period 2004 to 2009. Relevant articles were selected and supplemented with appropriate articles from their references. This three-part series thus reviews the current knowledge of nitrous oxide. In this, the first article of the series, the history and basic science are reviewed. The focus is on the latest knowledge regarding mechanisms of action and possible pathophysiological mechanisms, and the clinical relevance thereof. New considerations regarding pre-operative assessment and premedication are also presented. The remainder of the series will deal with the clinical controversies surrounding nitrous oxide, analyse claimed risks and benefits, and discuss its role in 2010.

History

Nitrous oxide is the "venerable old gentleman" of the anaesthetic world. Its long, eventful and often controversial "life" began in 1772. It was in this year that nitrous oxide was first synthesised by Joseph Priestley, clergyman and discoverer of many "airs" or gases, including oxygen.[1,2,3] In 1799, while conducting research into the role of these so-called "airs" in spreading disease, Humphrey Davey inhaled nitrous oxide and made an observation that, if heeded, could have prevented half a century of needless surgical suffering.Noticing that it eased the pain of his erupting wisdom tooth, he wrote: "As nitrous oxide…appears capable of destroying physical pain, it may probably be used with advantage during surgical operations".[1,4]

After such a promising "childhood", nitrous oxide experienced a rebellious "adolescence". Its chief use was in nitrous frolics and public demonstrations. Here it gained the moniker "laughing gas" for the amusement it generated in those watching the intoxicated volunteers.

Interestingly, Samuel Colt used the money he earned as a nitrous oxide peddler to finance the development of his Colt six-shooter, perhaps the best known weapon of all time.[4]

This "misspent youth" was not entirely wasted, however. It was at one of these public displays, in 1844, that Horace Wells, a dental surgeon, had an epiphany. As he watched, a volunteer accidentally cut his leg while under the influence of nitrous oxide. The man appeared to feel no pain, and Wells wondered if the gas would allow painless dental extractions or amputations.[1,5] The following day, Wells had one of his own teeth extracted without pain or adverse effect[1]. Filled with enthusiasm, he arranged a demonstration at Harvard Medical School in January 1845. The subject groaned and moved while undergoing a dental extraction and the demonstration was viewed as a failure, although the patient later claimed to have felt very little.[1,3,4] Discredited, Wells withdrew from the development of nitrous oxide and committed suicide three years later.[6,7]

After another long period in the shadows, nitrous oxide reappeared in 1863. In this year, Gardner Colton, who had given the demonstration attended by Wells in 1844, resumed his laughing gas shows, and successfully administered a dental anaesthetic with nitrous oxide. Soon afterwards, the Colton Dental Association was established and achieved great popularity. All of this early activity had occurred in America. The worldwide spread of nitrous oxide anaesthesia began in 1867 when Colton successfully demonstrated its use at the First National Congress of Medicine in Paris. It subsequently became incorporated into obstetric

practice for labour analgesia and, as predicted by Wells, found a valuable role in the operating room.[1,4]

Nitrous oxide has, however, had a most eventful "adult life", with great triumphs being interspersed with dark controversy. Our knowledge of the physics and pharmacology of nitrous oxide has grown enormously, from the belief that hypoxia was central to its mechanism of action and that cyanosis, lividity and clonic movements of the limbs, secondary to severe hypoxia, were a regular accompaniment to its use.[1,4,8]

We have witnessed nitrous oxide being lauded as an essential component of almost every general anaesthetic. We have also, increasingly, seen wave after wave of scandal: hypoxic events, neurological complications, foetal loss… particularly now as new pretenders to the throne try to unseat it.

What is the truth, or the nearest we have to it (hard science), and what is a fairy tale?

Basic science

In order to fully appreciate the issues surrounding nitrous oxide, a sound understanding of its physicochemical properties and pharmacological actions is required.

Physicochemical properties

Nitrous oxide is a colourless, non-irritant, odourless, inorganic gas with a molecular weight of 44. It has a boiling point of -88,5 °C, and a critical temperature of 36,5 °C. It is thus a gas at room temperature and pressure, but can be stored as a liquid under pressure. Although non-flammable and non-explosive, nitrous oxide supports combustion well.

The minimum alveolar concentration (MAC) of nitrous oxide is 104%, and it is thus not suitable as a sole anaesthetic agent except theoretically, under hyperbaric conditions.[3, 9, 10]

Nitrous oxide is relatively insoluble when compared to the volatile anaesthetic agents. This leads to one of the key characteristics of nitrous oxide: its very rapid onset and offset of action. Only desflurane has a lower blood/gas partition coefficient. However, its fat/blood partition coefficient is over 10 times that of nitrous oxide.

Nitrous oxide is, however, far more soluble than nitrogen and, as a result, nitrous oxide diffuses into air-filled spaces far more rapidly than nitrogen can diffuse out (clinically relevant because of the high concentrations used compared with volatiles). This leads to the expansion of the space if compliant or, if poorly compliant, to a rise in pressure. Thus, nitrous oxide may lead to the expansion of a pneumothorax, air embolus, or bowel. It may also increase intracranial pressure in the presence of a pneumocephalus, intraocular pressure when intraocular gas injections have been performed, middle ear pressure, and cuff pressures in endotracheal tubes and supraglottic airways devices.

A similar process may lead to diffusion hypoxia.

Two other phenomena related to the physicochemical properties of nitrous oxide are the concentration effect and the second gas effect.[10] The concentration effect refers to the increase in the rate of increase of the alveolar concentration of nitrous oxide with increasing inspired concentration, i.e. increasing the inspired concentration of nitrous oxide will disproportionately augment its own uptake. This is as a result of two processes: the concentrating effect and the augmented inflow effect, which are discussed in most standard texts. The concentration effect of nitrous oxide will also augment the uptake of concomitantly administered volatile agents: this is known as the second gas effect. It is one explanation for more rapid gas induction when used in conjunction with a volatile agent. It is, however, probably not clinically very important, the supplementary anaesthetic effects of nitrous oxide being more relevant.

Table I. Partition coefficients of inhalational anaesthetics at 37 °C

Agent	Blood/Gas	Brain/Blood	Muscle/Blood	Fat/Blood
Nitrous Oxide	0.47	1.1	1.2	2.3
Desflurane	0.45	1.3	2.0	27
Halothane	2.5	1.9	3.4	51
Isoflurane	1.4	1.6	2.9	45
Sevoflurane	0.65	1.7	3.1	48
Nitrogen	0.013			

Modified from Miller's Anesthesia, 6th Ed ; with permission from Elsevier[4].

These phenomena are traditionally viewed as being short-lived and only relevant to nitrous oxide and the volatile agents. However, Peyton et al described the persisting concentrating and second gas effects of nitrous oxide on oxygenation.[11] They found that, after a mean of 47,5 minutes of anaesthesia with 70% N_2O/30% O_2, PaO_2 rose by 1,8 kPa, compared to 70% N_2/30% O_2. This was only noted when there was significant ventilation/perfusion (V/Q) inhomogeneity, as occurs commonly during anaesthesia. The authors concluded that the perfusion-driven uptake of soluble (in relation to nitrogen and oxygen) gases, for example N_2O, occurs in lung units with a relatively low V/Q ratio. So, even after the phase of rapid uptake of nitrous oxide is completed, there is ongoing uptake from these low V/Q units, and thus ongoing concentrating and second gas effects on alveolar PO_2. As these lung segments receive a large proportion of pulmonary blood flow, the effect on arterial PO_2 is significant and is greater than the competing effect of absorption atelectasis, which is also significant with nitrous oxide.

Mechanisms of action

Although some controversies remain, we have come a long way from the days when hypoxia was thought to be the key to the mechanism of action of nitrous oxide. As can be seen from the table below, nitrous oxide exhibits many similarities to ketamine and xenon, with regard to mechanism of action and clinical features, and differs substantially from both the intravenous anaesthetic agents and the volatile anaesthetics.[12]

The sedative and immobilising effects of nitrous oxide appear to be mediated separately to its analgesic effects, and will thus be discussed individually.[12-16] This is not only important conceptually but may have practical implications, as discussed later.

Sedative/amnestic/immobilising mechanisms

Central to the mechanism of the nitrous oxide anaesthetic action appears to be the noncompetitive inhibition of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor. Glutamate is the major excitatory neurotransmitter in the brain. N_2O thus markedly inhibits glutamate-mediated excitatory neuro-transmission. Whether this effect is partially mediated by other glutamate receptors (i.e. AMPA and kainite), or not, is currently not known.

Inhibition of these receptors alone is, however, insufficient to fully explain nitrous oxide's anaesthetic actions. Also important appears to be the two-pore domain potassium channels, in particular TREK-1. This is a background leak potassium channel that regulates the resting membrane potential in neurons of the brain and spinal cord. When activated, these channels open, resulting in increased potassium conductance/efflux, hyperpolarisation and decreased excitability of the neurons.

Inhibition of the $\alpha_4\beta_2$ neuronal nicotinic acetylcholine receptor may contribute to the amnestic action of nitrous oxide.

What is important to note is that, unlike the IV and volatile agents, $GABA_A$ receptor enhancement does not occur as part of nitrous oxide-induced anaesthesia.

Table II. General anaesthetic classification based on clinical features and molecular targets

	Group 1	Group 2	Group 3
General anaesthetics	Etomidate, propofol	Nitrous oxide, ketamine, xenon	Halogenated ethers (e.g. isoflurane, desflurane) and alkanes (e.g. halothane)
Clinical features	Strong hypnotics Strong amnestics Weak immobilisers Slow cortical EEG	Weak hypnotics Weak immobilisers Potent analgesics No EEG slowing	Strong hypnotics Strong amnestics Strong immobilisers Slow cortical EEG
Ratio of MAC-immobility to MAC-awake	4 (propofol)	1,5 (N2O) 2 (Xe)	2 (halothane) 3 (halogenated ethers)
Molecular targets	$GABA_A$ receptors (β_3 and β_2 subunits)	NMDA receptors AMPA receptors Neuronal nAChRs 2-pore K^+ channels	$GABA_A$ receptors Glycine receptors Glutamate receptors (NMDA and AMPA) Neuronal nAChRs 2-pore K^+ channels

EEG = electroencephalogram; NMDA = N-methyl-D-aspartate; $GABA_A$ = Υ-aminobutyric acid subtype A; AMPA = a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid. Modified from Solt and Forman; with permission from Wolters Kluwer Health.[12]

Figure 1. Analgesic mechanism of action

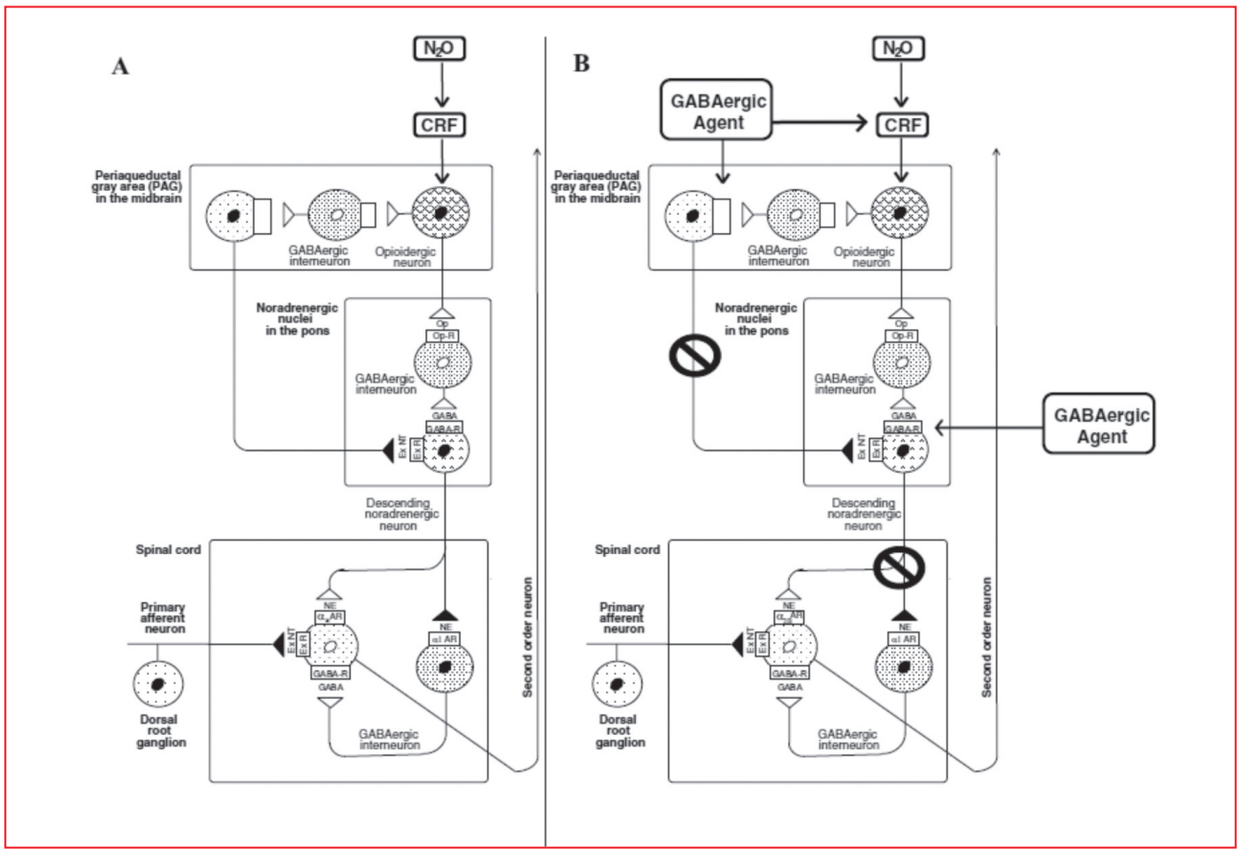


Figure 1. (A) Neuronal pathways for nitrous oxide-mediated analgesia. (B) Effects of addition of a GABAergic agent. "No entry" sign = GABA-mediated prevention of activation of noradrenergic descending inhibitory neurons by nitrous oxide. Black triangle = excitatory synapse; white triangle = inhibitory synapse; black oval = nucleus of an active cell; white oval = nucleus of an inactive cell. AR = adrenoreceptor; ExNT = excitatory neurotransmitters; ExR = receptors for excitatory neurotransmitters; GABA = ϒ-aminobutyric acid; GABA-R= ϒ-aminobutyric acid receptor; LC = locus ceruleus; NE = norepinephrine; Op = opioid peptides; Op-R = opioid receptor. Reprinted with permission from Wolters Kluwer Health[13].

Nitrous oxide-induced analgesia is initiated supraspinally. A key initiating event appears to be the release of corticotrophin-releasing factor (CRF) from the hypothalamus, as a result of NMDA-receptor antagonism. Activation of opioidergic neurons in the peri-aqueductal grey matter (PAG) and noradrenergic neurons in the locus ceruleus, A5 and A7 areas in the brainstem then follows. Specifically, CRF causes release of opioid peptides in the PAG. These activate opioid receptors on GABAergic interneurons, inhibiting these inhibitory interneurons. Recent evidence also points to a role for the nociceptin receptor ("opioid-like") and its endogenous ligand, nociceptin.[15] This system works via either direct dopamine antagonism, or indirectly via GABA inhibition.

The end result of the above mechanisms is the removal of the GABA-mediated inhibition of the excitatory interneurons of the descending inhibitory noradrenergic neurons of the pons/medulla. Simply put, one now has activation of the descending noradrenergic pathways, which release noradrenaline (NA) at their nerve terminals in the dorsal horn of the spinal cord. NA then activates α_1 receptors on GABAergic interneurons, increasing GABA release, which in turn activates inhibitory $GABA_A$ receptors on second order afferent neurons. In addition, there is activation of postsynaptic α_{2B}-receptors on the second order neurons. The nett effect is inhibition of the second order neurons and a reduction in the ascending transmission of pain impulses to the brain.

It is interesting to note, as shown in (B) of figure 2, that addition of a GABAergic agent can attenuate the nitrous oxide-induced activation of the noradrenergic descending inhibitory interneurons. In addition, animal studies have shown that GABAergic agents, e.g. volatiles and propofol, reduce nitrous oxide's

analgesic effect. The clinical evidence to back this up is poor but it has led some authorities to state that its analgesic effect, when co-administered with a volatile, is minimal and is not critical to its anaesthetic action. The clinical correlate of this view is that nitrous oxide is a potent analgesic when administered alone, e.g. for procedural sedation, but when administered as part of a balanced anaesthetic the analgesic effect is reduced and may, in fact, be minimal. This view is not in accordance with an overwhelming number of publications that attest to its analgesic efficacy, even when used as part of a balanced anaesthetic technique. As an example, Mathews et al equated a remifentanil whole blood concentration of 2 ng/ml with 66% N_2O, hardly insignificant.[17]

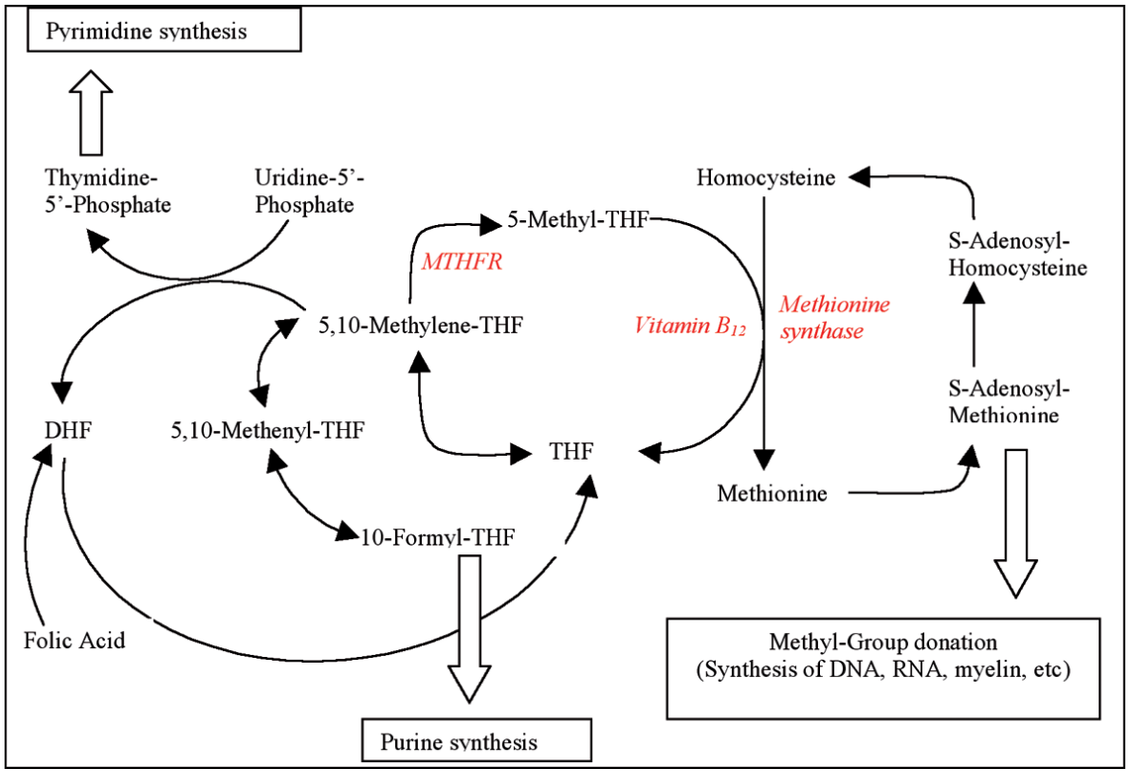
Also of interest, and a topic of some controversy, is the interaction between N_2O and opioids. The review by Sanders noted two studies that suggest that, if sufficient fentanyl is given, the addition of nitrous oxide does not have any further MAC-reducing effect[13]. In contrast to this, the same review refers to a study that noted a 60% MAC reduction in sevoflurane when nitrous oxide was added to a sevoflurane-remifentanil anaesthetic. The authors felt that this may be because nitrous oxide's NMDA receptor antagonism counteracts the potentiation of NMDA-receptors by remifentanil (not seen with fentanyl), and thus continues to be MAC sparing when used with remifentanil but not with fentanyl. It is also thought that this mechanism may allow nitrous oxide to block remifentanil-induced postoperative hyperalgesia.

So, is the role of nitrous oxide interchangeable with that of the short-acting opioids, or are there important differences? Many questions remain. It is clear, however, that the interaction between nitrous oxide and the volatiles and opioids is more complex than frequently realised.

Pathophysiological mechanisms

Inhibition of methionine synthase by nitrous oxide is central to the pathophysiology of many of its claimed adverse effects, and will thus be discussed in some detail[2, 13, 18, 19 - 21]. The pathophysiology of the adverse effects resulting from the physicochemical profile of nitrous oxide (e.g. expansion of air-filled spaces) has been alluded to previously and will not be discussed further here.

Figure 2. Possbile pathophysiological mechanisms related to methionine synthase inhibition. Note role of vitamin B_{12} and folate as key cofactors.



DHF = dihydrofolate; MTHFR = 5,10-methylene-THF reductase; THF = tetrahydrofolate. Modified from Weimann; with permission from Elsevier[22].

Nitrous oxide irreversibly oxides the cobalt I (Co^{+}) form of cobalamin (vitamin B_{12}) to the cobalt III (Co^{3+}) and cobalt II (Co^{2+}) forms. Oxidised cobalamin is no longer biologically active and can thus no longer act as the cofactor for methionine synthase, leading to its inhibition. As can be seen from the figure, methionine synthase is central to the generation of methionine, and hence S-adenosyl methionine, from homocysteine. S-adenosyl methionine is the methyl donor in over 100 metabolic steps, including the methylation of DNA, RNA, proteins, phospholipids, myelin, polysaccharides, cathecholamines and neurotransmitters. Inhibition of methionine synthase will thus result in a reduced concentration of methionine and an elevated concentration of its precursor homocysteine.

Methionine synthase also requires 5-methylene-tetrahydrofolate (5-methylene-THF) as a cofactor. Tetrahydrofolate (THF) is methylated, via methyl groups donated from the interconversion of serine and glycine, to 5,10-methylene-THF. The enzyme 5,10-methylene-THF reductase (MTHFR) then reduces 5,10-methylene-THF to 5-methylene-THF, which in turn donates a methyl group to cobalamin, forming methylcobalamin and regenerating THF. This process is central to the broader folate cycle which is crucial for purine and pyrimidine synthesis. Methylcobalamin is the final methyl donor for methionine synthase.

Folate-cobalamin cycle inactivation has been proposed to be responsible for adverse effects of nitrous oxide as diverse as megaloblastic anaemia, neurotoxicity (including subacute combined degeneration of the spinal cord), immunosuppression, impaired wound healing, and teratogenicity. The elevated homocysteine has been linked to endothelial dysfunction and hypercoagulability, and the consequences thereof e.g. peri-operative adverse cardiac events and venous thrombo-embolism. In addition, hyperhomocysteinaemia has been associated with the development of atherosclerosis and its consequences, neurodegenerative diseases/dementia, and potentiation of excitotoxicity.[13, 19, 20, 24]

What is the clinical relevance of these changes? There are a number of factors to consider.

Firstly, what level of exposure is required to significantly inhibit the above pathways? Human data suggest that 70% nitrous oxide results in a 50% reduction in methionine synthase activity within 46 - 120 minutes, with almost no activity detectable after 200 minutes. Recovery appears to take place within 3 - 4 days.[13, 24]

Secondly, are all patients equally susceptible? It appears not. Most patients have adequate stores of S-adenosylmethionine to see them through the peri-operative period[19]. At risk patients include those who are susceptible to vitamin B_{12} or folate deficiency, and those with certain genetic profiles.

Table III. Risk factors for vitamin B_{12} or folate deficiency[13, 23, 25]

Inadequate intake	Vegans
	Elderly
	Alcoholics
	Poverty
Malabsorption: *Gastric*	Pernicious anaemia
	Gastrectomy
	Achlorhydria
	Antacids
Malabsorption: *Ileal*	Sprue
	Resection
	Granulomatous disorders e.g. Crohn's disease
	Bacterial overgrowth
	Intestinal parasites
Increased Requirements	Pregnancy
	Malignancy
	Infancy
Multifactorial	Critically ill

Certain rare genetic disorders have been associated with adverse outcomes following nitrous oxide anaesthesia, e.g. autosomal recessive MTHFR deficiency/type III homocystinuria. These are exceedingly rare, and arguably not really relevant in routine clinical practice. What could be far more relevant, however, are certain fairly recently described, relatively common single nucleotide polymorphisms (SNPs) of the MTHFR gene. The 677 cytosine-thymidine (677C > T) and 1 298 adenosine-cytosine (1 298A > C) SNP result in reduced MTHFR activity. Homozygosity for these mutations results in higher baseline plasma homocysteine levels and greater postoperative increases after nitrous oxide exposure compared to both wild-type and heterozygous patients. The clinical correlates of this difference are, as yet, unknown. These reports are of interest, however, as these mutations are common, with approximately 20% of the Western European population being homozygous for one of the mutations. It must also be pointed out, though, that many non-genetic factors influence homocysteine levels, including drugs (antibiotics, isoniazid, antiepileptics) and medical conditions (hypothyroidism).[2, 13, 18, 19]

Thirdly, are any adverse effects time-dependent? The answer is "yes" in some circumstances. Subacute combined degeneration of the cord, for example, is almost exclusively described in long-term nitrous oxide use/abuse. It is not, however, as clear in other situations. Elevated homocysteine levels appear to result in endothelial dysfunction and hypercoagulability in the acute setting. Chronic hyperhomocysteinaemia is associated with atherosclerosis and chronic neurological disease e.g. dementia. The exact time scale and range of effects, if any, of elevated homocysteine in the peri-operative setting is not currently known. It is not even known if the elevated homocysteine is causative or if it is merely an "innocent bystander".[2, 13, 14, 20, 25]

Finally, can these potentially adverse biological effects be prevented? It appears that they can. Badner et al showed that pre-operative supplementation with folate, B_6 and B_{12}, for a week prior to orthopaedic surgery, prevented the nitrous oxide-induced post-operative increase in plasma homocysteine[21]. This obviously needs further study, for example to optimise the timing and duration of supplementation, but is an interesting prospect.

Conclusion

Our knowledge of nitrous oxide has advanced beyond anything Priestley could possibly have conceived of in 1792. It is clear though that much remains to be learnt regarding nitrous oxide's mechanisms of action and pathophysiological mechanisms, and the relevance of these to routine clinical practice. Some fascinating concepts are beginning to come to the fore. We should probably be evaluating a patient's risk of B_{12}/folate deficiency as part of our pre-operative assessment; and surely the pre-operative assessment of the future has to include genetic profiling, with MTHFR SNP's being an appropriate example. It also raises the question of whether vitamin supplementation should be part of our premedication, or postoperative therapy, if we are considering nitrous oxide as part of our anaesthetic regime. With this background in mind, the second and third parts of this series will take an evidence-based look at the claimed risks and benefits of nitrous oxide.

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