Nitrous Oxide Abuse

Subjects: Clinical Neurology

Contributor: Emeline Gernez, Graham Robert Lee, Jean-Paul Niguet, Farid Zerimech, Anas Bennis, Guillaume Grzych

The recreational use of nitrous oxide (N_2O), also called laughing gas, has increased significantly in recent years. In 2022, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) recognized it as one of the most prevalent psychoactive substances used in Europe. Chronic nitrous oxide (N_2O) exposure can lead to various clinical manifestations. The most frequent symptoms are neurological (sensitive or motor disorders), but there are also other manifestations like psychiatric manifestations or cardiovascular disorders (thrombosis events). N_2O also affects various neurotransmitter systems, leading to its anesthetic, analgesic, anxiolytic and antidepressant properties. N_2O is very challenging to measure in biological matrices. Thus, in cases of N_2O intoxication, indirect biomarkers such as vitamin B12, plasma homocysteine and plasma MMA should be explored for diagnosis and assessment.

Keywords: nitrous oxide ; cobalamin ; homocysteine ; methylmalonic acid ; vitamin

1. Introduction

The recreational use of nitrous oxide (N_2O), also called laughing gas, has increased significantly in recent years. In 2022, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) recognized it as one of the most prevalent psychoactive substance used in Europe [1]. N_2O is typically consumed by inhalation from balloons for its euphoric properties, which last only a few minutes. However, this consumption is associated with a risk of both acute and chronic toxicity. Chronic toxicity is rising in recent years because of an increase in consumption rates (in terms of quantity and frequency) related to the dependence and the tolerance effect of this molecule.

2. Clinical Manifestations

2.1. Brief History of the First Reported Manifestations of Chronic N_2O Exposure

The first adverse effects of N_2O, reported in 1952, were hematological in nature described in young patients infected with tetanus to relieve their pain. One of the patients, a 15-year-old boy, died days later from septicemia and granulocytopenia secondary to severe bone marrow depression [2]. Several years later, one of the authors coined this incident as the time “when nitrous oxide lost its innocence” [3].

The first neurological adverse effects were reported in 1978 by Robert Layzer in San Francisco, even without Magnetic Resonance Imaging (MRI) and biological background [4][5]. Many case reports have since been described and the number of patients drastically increasing over time. A small increase in publications and cases were reported in the late 90′s due to an increased recreational use of N_2O among dentists and healthcare givers in the USA who had easy access to the gas [6].

2.2. Common Symptoms and Signs

As reported initially by Layzer, patients had a mixture of central and peripheral nervous system symptoms (**Figure 1**). In several examinations, subjects even alternated between hyper and hyporeflexia, as myelopathic or neuropathic influences predominated. This presentation was called “myeloneuropathy” [4].

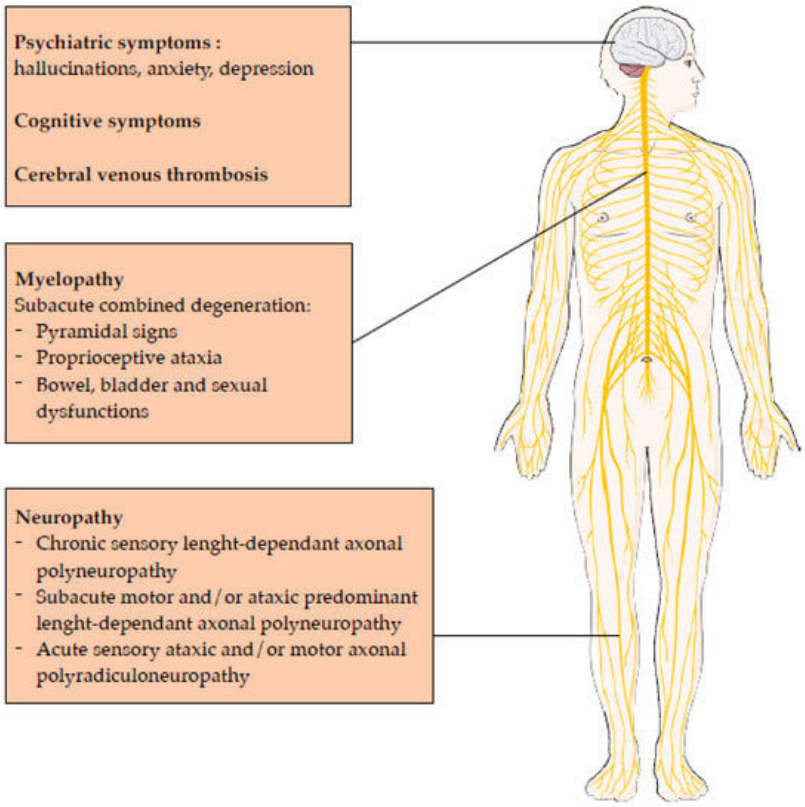

Figure 1. Clinical manifestations related to nitrous oxide intoxication.

Chronic N_2O users have common mild neuropathic symptoms. A large survey conducted with more than 240,000 people concluded that 17% of participants indicated a chronic use of N_2O, with 4.2% of recreational users reporting persistent paresthesia or numbness, distributed in a stocking glove pattern, suggestive of a length-dependent axonal polyneuropathy, as seen in many others toxic neuropathies [7].

2.3. Central Nervous System Involvement

The classical spinal magnetic resonance presentation is a hypersignal on sagittal T2-weighted images in the cervical level, and to a lesser extent, in the thoracic level. An inverted “v” shaped signal is observed on the axial plane, corresponding to the dorsal columns. Interestingly, these lesions are rarely responsible for a spinal sensory level on examination. In a Chinese study describing 15 patients with spinal cord lesions, 80% had a cervical lesion and 86.7% did not have a spinal sensory level on clinical examination. This may be explained by their non-transverse nature. The vulnerability of the cervical segment had been attributed to the higher density of myelinated fibers of the dorsal columns in the cervical segment compared with the thoracic segment [8].

2.4. Peripheral Nervous System Involvement

In the first report from Layzer in 1978, the electromyographic (EMG) examination showed normal to subnormal sensory conduction studies, with predominantly abnormal motor conduction studies. It was concluded that neuropathy was due to axonal degeneration rather than to segmental demyelination. One patient had a sural-nerve biopsy that showed only non-specific axonal degeneration [5].

Many N_2O abusers have persistent paresthesia or numbness distributed in a stocking-glove pattern, suggesting a length-dependent axonal polyneuropathy. Patients have predominantly lower limb motor axonal injury, consisting of a motor length-dependent axonal polyneuropathy, sometimes associated with demyelinating injury in the upper limbs [7]. From an electrophysiological perspective [9], there seem to be specific features not usually seen in classic length-dependent polyneuropathies, such as:

- More motor and sensory nerve injury in the lower limbs compared to the upper limbs,
- More motor nerve injury than sensory nerve injury in the lower limbs,
- More demyelinating features in the sensory and motor nerves of the upper limbs, with a marked motor predominance.

2.5. Prognosis of Central and Neurological Nervous System Involvement

Data regarding the long-term prognosis of neurological consequences of N_2O consumption is scarce. Many patients do not come back to their follow up medical appointment. A French study reported the evolution of 6 patients after a mean time of 4.9 months (interquartile range 3.0–6.25). They evaluated their Overall Neuropathy Limitations Scale, which measures disability due to peripheral neuropathy, and found that they have a score of 2 on the lower limbs on second evaluation, corresponding to an independent but abnormal looking gait [10].

3. Pharmacological Effects

3.1. Dependence Producing Potential of Nitrous Oxide

This short-lived psychoactive effect of N_2O may precipitate frequent and heavy use. Coupled with this is N_2O's reinforcing effect [11][12][13], which is the ability of a drug to increase the probability that it will be self-administered again. The nucleus accumbens (NAcc), located in the basal forebrain, is involved in motivation, pleasure and reward. In the NAcc, antagonism of the NMDA (N-methyl-D-aspartate) subtype of glutamate receptors by N_2O causes disruption of glutamate homeostasis, which otherwise helps establish and maintain drug-seeking behavior [14]. Genetic variability at the receptor level is a potential factor affecting susceptibility to developing N_2O dependence [15].

N_2O is recognized for its anesthetic, analgesic, anxiolytic and anti-depressant effects [1][16] and the molecular targets of N_2O and concomitant regulation of key neurotransmitters (glutamate, opioid, noradrenaline and γ-aminobutyric acid (GABA) will be considered in turn with major neuropharmacological effects (**Figure 2**).

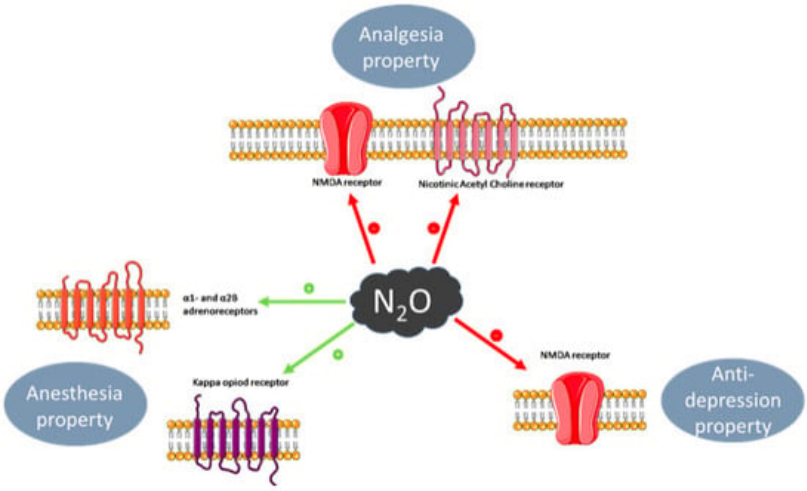

Figure 2. Main neurological effects of Nitrous Oxide, neurotransmitter modulation and receptor targets. Red arrow for inhibition and green arrow for activation.

3.2. Anaesthesia

N_2O administration is via inhalation utilizing a simple face mask, laryngeal mask airway or an endotracheal tube. In accordance with the European Society of Anesthesiology Task Force on Nitrous Oxide, N_2O is used at lower concentrations (30 to 50% with oxygen) for sedation in surgical and dental procedures and up 70% for general anesthesia with associated unconsciousness and immobility [17]. N_2O is the least potent inhalational anesthetic, as defined by the minimum alveolar concentration (MAC), which prevents a movement response (immobility) during a painful (e.g., surgical) stimulus. Currently, non-competitive inhibition of the NMDA receptors, specifically the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainite forms, is considered the main molecular target for N_2O's anesthetic effect [18]. These receptors comprise ligand-gated ion channels that are activated by glutamate.

3.3. Analgesia

Analgesia is defined as insensibility to pain without loss of consciousness, and a property of general anesthesia [17]. The analgesic and anti-nociceptive effect of N_2O involves the opioidergic system, by antagonism of the kappa opioid receptor, and the subsequent regulation of GABAergic and noradrenergic systems. In the periaqueductal grey (PAG) area of the midbrain, which is responsible for modulation of descending pain, blockade of these opioid receptors ablates nitrous-oxide-mediated analgesia, itself also partially reversed by the opioid receptor antagonist naloxone [19]. Corticotrophin releasing factor from the hypothalamus is also released in response to N_2O [20] and causes activation of opiodergic

neurons in the PAG with release of endogenous opioids such as dynorphins, which also activate kappa opioid receptors [21].

3.4. Anxiolytic Effect

Anxiolytics are used to prevent or treat anxiety symptoms or disorders and include the benzodiazepine class of drugs. The anxiolytic effect of N_2O involves activation of the gamma-aminobutyric acid type A (GABAA) receptor through its benzodiazepine binding site, though a direct effect is uncertain [22][23]. However, any such effect is considered minimal compared to the effect on NMDA receptors [24].

3.5. Anti-depressant Effect

N_2O's purported anti-depressant effect [25], which is a comparatively more recent and ongoing area of exploration, is mediated through non-competitive inhibition of NMDA receptors, and is considered analogous to that of ketamine and similarly short-lived [25][26]. The latter property perhaps hinders its use clinically as an anti-depressant. Other purported molecular targets and effects include the regulation of Brain-derived neurotrophic factor (BDNF) which has a role in synaptic plasticity, synaptogenesis and neurogenesis [27][28], contributing to its anti-depressant effect, as opposed to neuronal atrophy and synaptic loss, as seen with stress and depression.

4. Laboratory Medicine

4.1. Direct N_2O Measurement

N_2O may affect driving behavior and may cause fatal car accidents. As such, detection is an important issue. N_2O has a very short half-life of a few minutes [29], as the uptake and elimination curves are comparable [30]. N_2O elimination is mainly pulmonary. When exposure ends, exhaled air concentration declines rapidly, from 66–70% to 6–9% at 5 min and to 2–4% at 30 min during normoventilation. The elimination is slower in cases of hypoventilation [31]. Thus, the measurement of N_2O in exhaled air is not routinely usable for patients presenting to the emergency department due to the time gap between consumption and admission. The issue is the same for toxicology screening: indeed, for police roadside controls, this appears to be difficult due to the time gap between arrest and sample collection.

Additionally, there are technical difficulties concerning N_2O measurement in biological fluids. First, gas chromatography–mass spectrometry (GC-MS) can be used, but has limitations, including the challenge of finding an optimal internal standard, the lack of sensitivity and the potential risk of leaks during sampling, extraction and analysis. Headspace-GC-MS, which is a method in which the sample is placed in a hermetically-sealed, gas-tight container, could be promising but need further studies to be used in laboratory medicine [32]. Infrared Spectroscopy techniques are sensitive methods to measure N_2O in air, but not on biological matrices [33].

4.2. Impact on Metabolism

4.2.1. Cobalamin and One Carbon Metabolism

The clinical presentation of N_2O intoxication is related to the functional impairment of vitamin B12, also called cobalamin (**Figure 3**). Indeed, N_2O is a powerful oxidant agent: it leads to the oxidation of the cobalt ion of cobalamin(I) [34], resulting in the formation of cobalamin(II), unable to accept methyl groups. This results in a decrease in the formation of methylcobalamin, which is a cofactor for methionine synthase (MS or MTR).

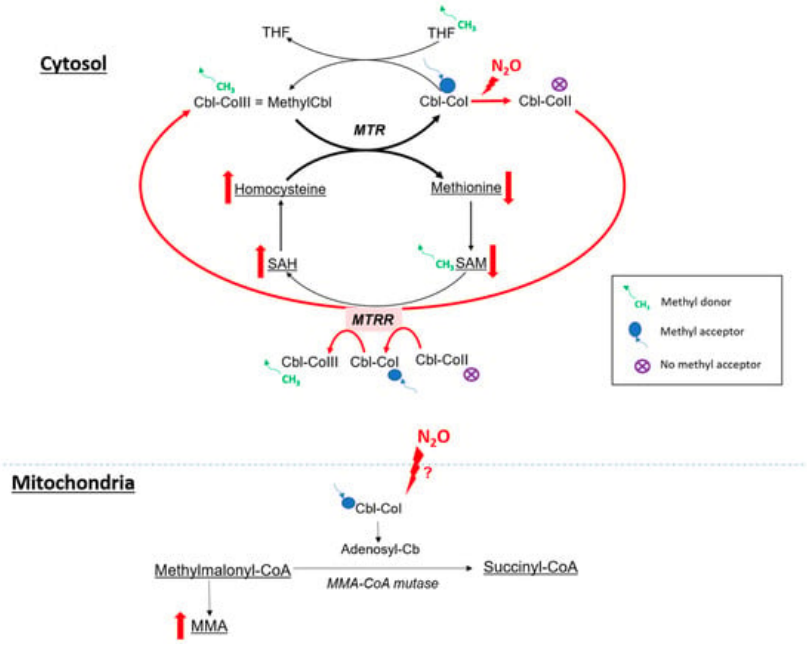

Figure 3. Hypothetic impact of N_2O on metabolism. SAH: S-adenosyl-homocysteine, SAM: S-adenosyl-methionine, MTR: methionine synthase, MTRR: methionine synthase reductase, THF: tetrahydrofolate, MMA: methylmalonic acid. Red arrow for major pathway in case of cobalamin oxidation.

4.2.2. N_2O and Oxidative Stress

N_2O has powerful oxidant properties. A study was conducted on 36 nurses occupationally exposed to anesthetics including N_2O during surgical procedures. Biological assessments revealed an increase in oxidative stress markers, including thiobarbituric acid-reactive substances (TBARS) and F2 isoprostanes. There was also a significant decrease in the activity of the antioxidant enzyme glutathione peroxidase (GPX), and an increase in the levels of reactive oxygen species (ROS) in peripheral blood leukocytes [35].

4.2.3. Homocysteine and Oxidative Stress

Hyperhomocysteinemia also enhances oxidative stress. In a study conducted on CBS (cystathionine beta-synthase) deficient mice [36], an inherited metabolic disease inducing severe hyperhomocysteinemia, several indicators of oxidative stress were notably increased. Lipid peroxidation markers, such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE), were elevated, as well as protein-associated carbonyl groups, indicating protein oxidation.

4.3. Indirect Biomarkers of N_2O Intoxication

4.3.1. Vitamin B12

As N_2O leads to a functional vitamin B12 deficiency, the quantitative deficiency in vitamin B12 is secondary and inconsistent [37]. Patients are also frequently supplemented with vitamin B12; increased levels of vitamin B12 can also be found in intoxicated patients. Consequently, it seems more pertinent to investigate functional markers of vitamin B12 in cases of N_2O intoxication, which are plasma MMA and plasma homocysteine.

4.3.2. Plasma Homocysteine

Plasma homocysteine is highly sensitive and can be used as a marker of recent N_2O consumption [37] because it rapidly increases in case of consumption. However, homocysteine levels decrease rapidly and can return to physiological values within several days after the last N_2O consumption [38]. This biomarker is also not specific to N_2O intoxication: plasma homocysteine increases in case of vitamin deficiency (vitamin B6, vitamin B9, vitamin B12), renal or hepatic injury, hypothyroidism and in certain metabolic diseases.

4.3.3. Plasma MMA

Plasma MMA is more specific than homocysteine in the exploration of vitamin B12 status because it does not depend of vitamin B6 and B9 status; but rise in case of renal insufficiency and in certain metabolic diseases. However, it is not a sensitive marker of N_2O abuse as its elevation is not consistent. Plasma MMA is correlated to the clinical severity [37]; thus, it can be used as a marker of clinical severity of N_2O intoxication.

4.3.4. Plasma Methionine

Methionine has also been investigated as a potential biomarker in nitrous oxide intoxication. Indeed, the decrease in MS activity could lead to a decrease in production of methionine, which is involved in the formation of myelin.

4.3.5. Oxidative Stress Markers

Oxidative stress markers could be of interest in N_2O intoxication. However, no studies have been conducted on patients with a recreational use but only on occupational exposure. Therefore, additional investigations are needed to determine whether these markers may be of interest as a consumption marker or as a marker of clinical severity.

4.3.6. Others Biological Parameters to Consider

Some biological parameters are crucial for the differential diagnosis of hyperhomocysteinemia, as this parameter is not specific of N_2O intoxication. Thus, renal (creatinine) and hepatic exploration (AST, ALT, alkaline phosphatase, GGT) should be performed, as well as vitamin assessment (vitamin B6, vitamin B9), to explore nutritional deficiencies [39]. Cell blood count can be performed to investigate a potential anemia, although N_2O does not appear to cause macrocytic anemia [40].

5. Conclusions

The recreational use of N_2O has seen a significant increase in recent years, leading to a growing concern about its acute and chronic toxicity. There is a wide range of chronic manifestations including myelopathy, neuropathy, psychiatric manifestations, cognitive symptoms and cardiovascular effects. N_2O interacts with neurotransmitter systems, leading to anesthetic, analgesic, anxiolytic and potential anti-depressant effects, with a potential dependance. Laboratory medicine plays a critical role in assessing N_2O intoxication, with biomarkers such as plasma homocysteine, a marker of recent consumption, and plasma MMA, a marker of clinical gravity. Other biomarkers, like oxidative stress markers, could be interesting but need further investigations.

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