Regulating the oxides of nitrogen – popping the myths

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Abstract

In 2015 and 2016, during the debates that culminated in The Psychoactive Substances Act 2016, both houses of the UK parliament debated the pharmacology of nitric oxide and amyl nitrites, otherwise known as “poppers”. The original draft Psychoactive Substances Bill had recommended that poppers should be made illegal. However, after strong opposition inside the Conservative party, the ruling party at the time, the government found a way to not ban them by claiming they were not psychoactive. Nitrous oxide, another recreational gas, was also a possible target of the Psychoactive Substances Bill but this was never explicitly mentioned in the debates. Once the Psychoactive Substances Act came into force the Crown Prosecution Service stated that nitrous oxide was psychoactive and so illegal to sell for psychoactive purposes. Many sellers of nitrous oxide canisters were arrested and some sent to prison. A series of Crown court prosecutions for nitrous oxide possession followed in which the Crown claim that nitrous oxide was psychoactive was challenged. In some of these the defendant was acquitted so making its current legal status uncertain. Here we delve into the pharmacology of nitric oxide and nitrous oxide and how the effects of these two gasses are related. A clear understanding of the pharmacology of these gasses is essential for the scientific underpinning of legislation which will directly impact on the life of recreational users and people involved in the selling of these substances.

Keywords

nitrous oxide, nitric oxide, poppers, laughing gas, alkyl nitrites

Introduction

In 2015 and 2016, probably for the first time ever, both houses of the UK parliament debated the pharmacology of a gas. This gas was nitric oxide, one of the three gaseous oxides of nitrogen. The other two being nitrous oxide (“laughing gas”, “nitrox”) and nitrogen dioxide (a toxin that’s a major air pollutant in cities produced by internal combustion engines).

Why was nitric oxide a topic of parliamentary debate? The answer is that it, or rather its precursor chemicals (alkyl nitrites or “poppers”) were being debated in reference to the 2015 Psychoactive Substances Bill. This Bill came into law in 2016 as the Psychoactive Substances Act 2016 and this Act defines psychoactivity thus: For the purposes of the Act, a substance produces a psychoactive effect if, ‘by stimulating or depressing the person’s central nervous system, it affects the person’s mental functioning or emotional state.’

The Psychoactive Substances Act 2016

This was a very unusual piece of legislation. For the first time ever in the UK substances were banned even if they were not harmful. The Act is prospective as well as retrospective so even psychoactive substances that have not yet been discovered will be illegal once they are made and sold. The Psychoactive Substances Act came into law on 27 May 2016 having been pushed through rapidly with little time for debate and despite its proponents agreeing on record that it was flawed in many ways. There was no clear definition of psychoactivity and the Act itself does not mention a single drug; there are no examples given. The three most popular drugs in the UK, alcohol tobacco and caffeine, were exempted from the Act despite according to expert opinion that alcohol the most harmful

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drug in the UK and tobacco a leading cause of death (Nutt et al., 2010). These exemptions were granted based on the grounds of precedence rather than safety. Disappointingly the duration of use required to meet the criterion of precedence was not defined.

The original draft Psychoactive Substances Bill had recommended that alkyl nitrite products should be made illegal. These are products such as amyl nitrite and isosorbide mononitrate that in the body release nitric oxide. Some for example amyl nitrite have been used recreationally by inhalation for over 150 years (Cheng, 2013) (well described in the book Fear and Loathing in Las Vegas by Hunter Thompson) whereas others have been developed more recently for the treatment of angina. Poppers are capsules that contain versions of these chemicals that are put into devices used as room deodorisers which slowly release them into the air to counter bad odours.

When these nitric oxide producing chemicals are deliberately ingested they release nitric oxide in the body causing relaxation of smooth muscle in blood vessels leading to vasodilation. This is why amyl nitrites are the standard treatments for cardiac chest pain (angina); by relaxing the arteries in the heart they enhance blood flow into it. Poppers are sold in small glass screwcapped bottles and the liquid inside is inhaled to get a brain rush or "high" as well as the other vasodilatory effects of nitric oxide particularly for sex. More liberated attitudes to male sexuality had led to increased use recently for sexual purposes. In recent years poppers have also become popular in the male gay scene. They relax sphincter muscles so to make anal intercourse easier and less likely to induce epithelial trauma (Romanelli et al., 2004).

During the debates on the Bill and in a remarkable act of openness, a member of the Conservative party opposed their own government’s plan to ban poppers because they themselves used them. They argued that banning poppers would increase harms from transmission of blood born viruses because anal sex would become more traumatic.

The Government needed a way out of this impasse. They decided that they now did not want poppers banned but, “as they were clearly psychoactive”, what could they do? They asked their expert Advisory Council on the Misuse of Drugs (the ACMD) to find them a way out. The ACMD solved the government’s problem by arguing that poppers were not psychoactive because they acted indirectly to change blood flow to the brain rather than acting on the brain directly. The ACMD argued that there was no evidence that nitric oxide acts on the brain and the only effect on the head is to cause terrible headaches (ACMD, 2016). This decision can be understood as follows. As detailed above the mechanism of action of poppers is via the production of the gas nitric oxide. This then acts to relax the (smooth) muscle tissue of blood vessels (a process called vasodilation) rather than to stimulate or depress the central nervous system. In other words, the effect of poppers is peripheral rather than on the central nervous system (ACMD, 2016).

As we describe later this ignores extensive evidence that nitric oxide is a neurotransmitter in the brain. However the claim it just worked on blood vessels got the Government off the hook and poppers were exempted from the Psychoactive Substances Act, to the delight of the gay community.

Nitrous oxide, another recreational gas, was also another possible target of the Psychoactive Substances Bill but this was never explicitly spoken as it was never mentioned in the debates. Use of nitrous oxide gas from inflated balloons had become popular amongst younger people in the previous few years as an alternative legal "high" to poppers and alcohol. Nitrous oxide was seen as being fun but with much improved safety compared with alcohol (Nutt et al., 2007; van Amsterdam et al., 2015).

The problem began once top footballers starting using it instead of alcohol at parties. Secret photos of well-known footballers inhaling a balloon of nitrous oxide were taken on mobile phones and then sold to newspapers. The Sun newspaper used these images to viciously attack these players even though what they were doing was perfectly legal, and much less likely to impair next-day playing performance than if they had used alcohol. A campaign was started to get nitrous oxide banned. But these populist papers realised that a scaremongering attack on laughing gas would be seen as ludicrous by the millions of people who had experienced it for pain control. So, a new name was invented—hippy crack – deliberately designed to scare the public and magnify its harms. By relating nitrous oxide consumption to crack cocaine without giving any background on the effects and possible harms of both substances implicates in stigmatisation of the former, only because it is also used as a recreational drug however with far less risks than the latter. As any experienced drug user or pharmacologist will confirm, nitrous oxide is as far removed from crack cocaine as codeine is from crystal meth.

But the newspaper campaign did what it was designed to do – it instigated a degree of moral panic about nitrous oxide use that politicians felt they needed to deal with. As the ACMD had only recently said that nitrous oxide was not sufficiently harmful to be controlled under the 1971 Misuse of Drugs Act (ACMD, 2016) the Psychoactive Substances Bill offered an alternative form of control. Though the harms or psychoactivity of nitrous oxide were never debated in the readings of the Bill, and indeed is not mentioned in the Hansard records of the debates, once the Psychoactive Substances Act came into force the Crown Prosecution Service stated that nitrous oxide was psychoactive and so illegal. Many sellers of nitrous oxide canisters were arrested and some sent to prison. A series of crown court cases followed that challenged, in some cases successfully, the Crown claim that nitrous oxide
was psychoactive. Currently it is not clear if it is legal or not.

Historical and scientific background to the two gases

Nitric oxide: NO is not inhaled as a gas but taken as organic nitrite compounds (e.g. amyl nitrites, colloquially called “poppers”) that immediately break down in the body to nitric oxide (Cederqvist et al., 1994). For over 150 years these substances have been used recreationally by some people to get a euphoric effect or “high” (Thompson, 1971). The effects of nitric oxide commonly include headache, dizziness, weakness, a feeling of warmth, and a loss of inhibitions. Visual hallucinations are reported and in extreme cases anaesthesia and coma can result (Davies et al., 2017).

These effects are believed due to the nitric oxide gas having a vasodilation effect (increasing blood flow) in the head. Nitric oxide also increases blood flow in some other organs including those involved in sexual activity which is why poppers are used by some to improve their enjoyment of these. This vasodilation is produced because nitric oxide relaxes the muscles that constrict the bore of blood vessels. Nitric oxide also relaxes other muscles including those involved in some sexual activities which is why, in recent years, poppers have also become popular in the gay scene as well as with those looking for a recreational drug.

There has been a few reports on poppers-induced methemoglobinemia when ingested orally. This is due to its direct hemoglobin-oxidizing effects (Lefevre et al., 2018; Tello et al., 2021). There are about 3 deaths per year from the use of nitric oxide in the form of poppers in the UK at present (ACMD, 2016).

Pharmacology of Nitric Oxide

Nitric oxide is a major signalling molecule and physiological mediator in the human body. It has a broad spectrum of action, with described roles in immunology (Nathan and Hibbs, 1991), vascular biology (Rapopport et al., 1983; Förstermann et al., 1986) and also serves as a neurotransmitter on the central and peripheral nervous system (O’Dell et al., 1991; Schuman and Madison, 1991). It can also regulate gene transcription (Khan et al., 1996; Gudi et al., 1999) and mRNA translation (Pantopoulos and Hentze, 1995; Liu et al., 2002) and produce post-translational modification of proteins (Pozdnyakov et al., 1993; Brune et al., 1994).

Nitric oxide is a chemically unstable molecule, with a half-life of 3–5s which can also be spontaneously inactivated in the presence of oxygen and the superoxide anion (Ignarro, 1989). Nitric oxide is highly lipophilic and readily permeates biological membranes.

In mammals, nitric oxide is predominantly synthesised by a family of enzymes called nitric oxide synthases (NOS). Different isoforms of these enzymes are found in different tissues: eNOS (endothelial), nNOS (neuronal), iNOS (inducible). All three isoforms use L-arginine as the substrate and oxygen and NADPH (nicotinamide-adenine-dinucleotide phosphate) as co-substrates ( Förstermann and Sessa, 2012 ).

More recently, a new mechanism has been described on the generation of nitric oxide under physiological hypoxic conditions, where nitrite and nitrate are reduced to form nitric oxide. Both pathways counteract since oxygen levels are determinant for the generation of nitric oxide by NOS whilst the nitrate-nitrite-nitric oxide pathway is enhanced when oxygen availability falls ( Lundberg et al., 2008 ).

As for its main physiological functions, when synthesised on the vascular endothelium, nitric oxide is responsible for the vasodilator tone which is essential for the regulation of blood pressure: drugs that blocking nitric oxide production led to a rise in blood pressure and drugs (e.g. sodium nitroprusside) that promote nitric oxide are used to lower blood pressure. Nitric oxide also acts as an inhibitor of platelet aggregation (Epstein et al., 1993).

Nitric oxide also act as a neuronal messenger. It modulates stimulation-evoked field excitatory potentials and firing rates from single neurons in almost all brain regions and on the spinal cord. Depending on the type of neuron and its location, different cellular functions have been found to mediate the main effect of nitric oxide. For example, ion channels can be modulated by nitric oxide-signalling products, such as cGMP and protein kinases. Nitric oxide through cGMP synthesis also reduces the function of γ-aminobutyric acid (GABA A) receptors in the cerebellum ( Zarri et al., 1994; Robello et al., 1996 ) and AMPA receptors in the cerebellum, forebrain and in the horizontal cells of the retina. (Dev and Morris, 1994; McMahon and Ponomareva, 1996).

Nitric oxide can also directly modulate neuronal function. In cortical neurons, nitric oxide transiently reduces GABA-mediated Cl- influx, enhancing excitability (Robello et al., 1996). It also enhances AMPA binding in slices of rat forebrain (Dev and Morris, 1994). Therefore in various brains regions, the same receptor can be modulated by nitric oxide via different mechanisms, depending whether this is a direct modulation by or indirect modulation by nitric oxide signalling products. It has also been demonstrated that nitric oxide is involved in memory tasks, such as olfactory and spatial memory, avoidance tasks, working memory and habituation to a novel environment. Nitric oxide-inhibitors tend to produce impairment of these capabilities and, for some of these tasks, the use of nitric oxide donors reverses the impairment provoked by the inhibitor. For a comprehensive review on this, see Prast and Philippu, 2001. In the peripheral nervous system nitric oxide also acts as a neurotransmitter of the
Nitric oxide has been approved for medical use in the United States and in Europe. It is used for the treatment of new-born infants with hypoxic respiratory failure associated with pulmonary hypertension in order to improve oxygenation. It is also approved to be used in conjunction to heart surgery aiming to decrease pulmonary arterial pressure and ultimately improving oxygenation (INOMax).

**Nitrous oxide: N₂O**

Nitrous oxide is a gas that was discovered over 200 years ago by UK chemists Priestley and Davy. Nitrous oxide was originally called “laughing gas” and has been used for several centuries as a hypoxic way to have physician's experience. In the 1840s its value as an analgesic was discovered and today it is still used in medicine for this purpose and as an anaesthetic. Currently in addition to its medical use, nitrous oxide is also used in catering as an inert propellant to froth up cream in food servings.

**Medical uses**

For the past 200 years nitrous oxide has been inhaled as a gas by hundreds of millions of people in medical settings for pain control. In this circumstance it is breathed, usually in a fixed combination mixture with oxygen to produce pain control. Subanaesthetic concentrations of nitrous oxide produce analgesic and anxiolytic effects without unconsciousness. Its use is very common and popular indications are for minor surgical operations, bone fracture re-setting and for the pains of childbirth. During this period there have been few reported adverse effects and nitrous oxide is globally recognised as a safe and effective analgesic medicine. This is why nitrous oxide is on the WHO essential medicines list (WHO List of Essential Medicines, accessed on 26/8/2021)

Nitrous oxide was one of the first drugs to be used in anaesthesia for surgery, with its first described used for this purpose dating back to the 19th century. Nitrous oxide has the most rapid onset of the inhalation agents and has a low blood-gas partition coefficient. It is transported in blood as a free gas and does not suffer biotransformation. Its elimination occur by expiration and, due to its minimal solubility, nitrous oxide is removed quickly from the body, making it a safe anaesthetic agents (Becker and Rosenberg, 2008). It has a limited potency and, because of that, it is usually used in to reduce the minimum alveolar concentration of a second inhalation agent for anaesthesia (e.g. halothane), in order to increase the rate of induction of the second gas (Epstein et al., 1964).

One benefit of nitrous oxide compared with other anaesthetic gases, is that it does not depress ventilation, rather it increases respiratory rate. But when combined with other sedatives, a more pronounced respiratory depression may occur. Subanaesthetic concentrations of nitrous oxide have little to no influence on cardiac output and heart rate. In higher concentrations, it may increase these variables.

Over the past decades there has been growing interest in the use of nitrous oxide for other medical purposes. One is in the treatment of depression that has failed to respond to conventional treatments (Nagele et al., 2021). Previously studies in South Africa have shown it can rapidly attenuate alcohol withdrawal and lead to improved mood and craving outcomes afterwards. Limited data suggest an impact on tobacco and opioid addiction also. The physiological mechanism for nitrous oxide anxiolysis appear to be independent to the analgesic effects (Gillman and Lichtigfeld, 1990).

**Recreational use**

This generally involves using a small metal canister (a whippit) that is used to froth cream in catering. This is opened and used to fill a balloon from which the gas is then inhaled. For a minute or two a state of quite extreme altered consciousness is produced, often with a euphoric release of mental tension that leads people to laugh. The effects wear off within a few minutes and full normal consciousness is restored.

It is this short–acting nature of nitrous oxide that makes it so appealing to users especially footballers (Mirror, 2018; talkSport, 2020; The Sun, 2020). The “high” of nitrous oxide dissipates in minutes whereas a comparable “high” from alcohol last hours and often leads to a hangover which is also impairing.

The much more enduring nature of alcohol intoxication means that users are vulnerable to accidents or assaults for many hours, whereas they are perfectly in control of their senses a few minutes after nitrous oxide so are less vulnerable and can even drive home safely. One other advantage of nitrous oxide is that when sold in whippets for food preparation the gas is pure. So unlike with most illegally-sourced recreational drugs users know what they are getting.

Because of the short duration of effects of nitrous oxide – just a couple of minutes – recreational users often use a number of canisters in a session, with over 17% of users reporting using 25 or more (Kaar et al., 2016) and examples of over 100 a day have been reported.

**Safety concerns**

Nitrous oxide has a wide margin of safety and it’s a valuable tool in dentistry, especially for children who can get...
easily agitated and anxious during a procedure. A survey with American paediatric dentists has shown that 61% of the respondents used nitrous oxide in their routine (Malamed, 1979). Another relevant data comes from a study that evaluated the therapeutic use of nitrous oxide in over 7000 patients in alcohol withdrawal. In conclusion, no significantly adverse effects or dependence production to nitrous oxide was found. (Gillman and Lichtigfeld, 1990).

Over the past two hundred years the recreational use of nitrous oxide has not been associated with significant harms. For example, in the ten years from 2002–2012 there were 9 nitrous oxide related deaths reported in the UK, about one per year. For comparison over the same decade deaths from alcohol were of the order of 50,000, that is about 5000 per year (data from the Office of National Statistics).

Heavy chronic use can be associated with vitamin B12 deficiency and neurological consequences from this (van Amsterdam et al., 2015).

In comparison with other recreational drugs nitrous oxide scores low on ratings of risk and harms (Nutt et al., 2007). This comparative safety is presumably the reason the ACMD in 2016 did not recommend that it be controlled under the MDAAct1971.

**Mechanism of action**

How nitrous oxide produces its medical effects on pain and mood is not yet fully understood but may involve alterations in blood flow in brain areas involved in pain and alertness as well as the neurochemical effects. Current evidence shows that nitrous oxide might have different mechanisms of action for analgesia, anxiolysis and anaesthesia and, as mentioned, these effects are dependent on the dose administered.

The mechanism of nitrous oxide anaesthesia is not fully known but current evidence suggests that it binds to proteins within neuronal membranes leading to a modification of ligand-gated channels and ultimately altering synaptic transmission. A recent study in humans shows that nitrous oxide also produces relaxation of blood vessels (vasodilation) in the brain (Dashdorj et al., 2013) and these changes in blood flow can explain the psychological effects of the gas.

Subanesthetic concentrations of nitrous oxide only produce analgesic effects without loss of consciousness (Dundee and Moore, 1960). There is strong evidence pointing towards an opioid-related mechanism for nitrous oxide-induced analgesia. Its antinociceptive effect can be completely blunted when lesioning the periaqueductal grey area (PAG) in the brain, a region where opioid receptors have an important role (Zuniga et al., 1987). Pharmacological inhibition with opioid antagonists via microinjection into the PAG also prevent nitrous oxide analgesia (Emmanouil et al., 2008). Interestingly, morphine-tolerant animals are also tolerant to nitrous oxide, however nitrous oxide-tolerant animals are not tolerant to morphine (Berkowitz et al., 1979). Taken together, these observations lead to the hypothesis that nitrous oxide could stimulate the release of opioid peptides, instead of desensitisation of opioid receptors - a well-known mechanism for tolerance from chronic opioid use. However the nature of these peptides and the subtypes of opioid receptors targeted are still under investigation.

As described above, nitric oxide acts as a neurotransmitter and also regulate the release of other neurotransmitters. There is evidence suggesting that nitric oxide also plays a role in nitrous oxide-induced analgesia. In mice, pre-treatment with AS-ODN, a selective inhibitor of neuronal nitric oxide synthase markedly reduced nitrous oxide induced antinociception (Li and Quock, 2001).

In mice, anxiolytic behavioural effects produced by treatment with nitrous oxide and benzodiazepines were equally effective and antagonised by flumazenil, a well-known benzodiazepine receptor blocker. Likewise, tolerance to benzodiazepines also inhibits nitrous-oxide-induced anxiolysis (Quock et al., 1992). These findings indicate that the anxiolytic effect produced by nitrous oxide may be associated with brain benzodiazepine receptor-related mechanisms. Since benzodiazepines work through enhancing endogenous GABA inhibitory mechanisms, further studies investigated the behavioural patterns of mice treated with nitrous oxide, chloridiazepoxide (a benzodiazepine), flumazenil and a GABAA receptor antagonist (SR-95531) in the light/dark exploration test. Treatment with nitrous oxide and benzodiazepines increased the time spent in the light compartment showing an anxiolytic effect. Pre-treatment with flumazenil or SR-95531 inhibited the anxiolytic effects evoked when nitrous oxide or chloridiazepoxide were given on their own.

Nitric oxide also seem to play a role in nitrous oxide-induced reductions in anxiety. In the light-dark exploration test with mice, selective nNOS inhibition prevented an increase on the time spent in the light from treatment with either nitrous oxide or chloridiazepoxide (Li and Quock, 2001). Also, when tested in the elevated plus maze in mice, both chloridiazepoxide and nitrous oxide treatments increased open-arm activity, an indicator of anxiolysis. This effect was blocked after treatment with a NOS inhibitor, an effect that could then be reversed by treatment with the NOS substrate L-arginine (Caton et al., 1994).

**Legal status under the Psychoactive Substances Act 2016**

The fact that poppers (and other sources of nitric oxide) were not specifically excluded from the Act (unlike alcohol and tobacco) means their status is not clear.
When used for medical purposes, catering and motor racing nitrous oxide is exempted from the Psychoactive Substances Act 2016.

When used recreationally for its psychoactive effect then nitrous oxide MAY be controlled under the Psychoactive Substances Act though in each case this has to be determined by a jury in a crown court. The key issue here is whether nitrous oxide produces its effects through stimulating or depressing the nervous system or through some other mechanisms.

Outstanding questions

The data we share in this review raise two important questions about the treatment of these two gases under the Psychoactive Substances Act 2016.

First - Should nitric oxide have been excluded from the Psychoactive Substances Act on the grounds of its acting as a vasodilator when it clearly is a major mediator of brain function and has for over a century been used to get "high"? Currently because the exclusion of nitric oxide is not written in law it is possible that prosecutions under the Psychoactive Substances Act 2016 could be enacted for the sale of poppers.

Second - Why, given the exclusion of nitric oxide was because of its vasodilatation effects, was not nitrous oxide treated in the same way as it too has powerful vasodilation effects in the brain?

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