Nitrous oxide and carbon dioxide: their similar and contrasting biological effects

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The similar and contrasting biological properties of CO_2 and N_2O appear to reflect fundamental biological processes in both very simple and the most complex organisms.

Introduction

A recent article in this journal[1] discussed the similarities and differences between nitrous oxide (N_2O) and carbon dioxide (CO_2) from a physico-chemical perspective. Here we consider the apparent similarities and differences between these two gases in a biological context.

It is well known that biologically active chemical agents can act directly with receptors in the brain to produce physiological and pharmacological effects.[2] These receptors have unique and specific physico-chemical structures and properties. Chemical substances that attach to these receptors do so by characteristic physico-chemical mechanisms, which then trigger a cascade of events through the interaction of the particular agent with the specific receptor.[2] It is therefore not surprising that the structural similarities of the N_2O and CO_2 molecules translate into similar biological actions. On the other hand, such structural and other differences as they have may account for their dissimilar biological effects.

Nitrogen and carbon cycles

Photorespiration involves the light-dependent evolution of CO_2 and uptake of oxygen in green leaves. During the 1950s and 1960s, the synthesis of glycolate by leaves in high O_2 concentrations or low CO_2 settings was demonstrated and the enzymic pathways involved were elucidated.[3] Ribulose bisphosphate carboxylase/oxygenase (rubisco, RuBP) is the primary enzyme involved, with both CO_2 and O_2 competing for its active site, the position on the enzyme to which the substrate binds. There is a well-established link between photorespiration and the nitrogen cycle in plants. During the decarboxylation of glycine, ammonia is produced in stoichiometric amounts with the CO_2 involved in photorespiration. Plants acquire their nitrogen either directly from the soil or through internal recycling of nitrate and ammonium. The amount of ammonium recyling in C_3 plants is significantly higher than in C_4 plants as a result of photorespiration. As the rate of ammonia production in C_3 plants can be 20 times the rate of primary nitrate assimilation, the plant cannot afford to lose this ammonia as a volatile gas but must have an effective mechanism for its re-assimilation. Three enzymes (glutamine synthetase, glutamate synthase and glutamate dehydrogenase) have been identified in transforming ammonia into amino acids but there is debate as to the role of the various oxidized nitrogen intermediates which are formed.[3] It is possible that one of these oxides is NO, which may be reduced to N_2O as has been shown in mammalian cells[4] and bacteria.[5] In support of this idea, plants have been shown to emit endogenously produced N_2O during nitrate assimilation.[6]

Nitrous oxide does not substitute for O_2 in either the cytochrome *c* oxidase or carbon monoxide (CO) diogenase reactions that produce, respectively, water and CO_2 and which are both catalysed by cytochrome *c* oxidase.[7] Rather, it acts by reducing the rate of electron transfer from cytochrome *c* to the O_2 reaction site.[7] It is therefore possible that N_2O interacts with CO_2 in the plant cell by reducing the flow of electrons to both CO_2 and O_2 reaction sites. The latter postulate is supported by the finding that N_2O inhibits the use of O_2 by plant tissue.[8]

The link between N_2O and CO_2 through the carbon and nitrogen cycles is also seen in the composition of the atmosphere and oceans during glacial cycles.[9] The end of a glacial cycle is marked by the outgassing of N_2O, a greenhouse gas, from sea water. This is described by a nonlinear model which takes into account the effect of variations in the ocean's content of fixed nitrogen following enhanced denitrification.[9] Outgassing leads to greater levels of atmospheric CO_2 during interglacial periods,[9] showing a direct relationship between CO_2 and N_2O in the atmosphere and ocean.

Nitrous oxide, like carbon dioxide, is produced endogenously

Nitrous oxide is a metabolic product of certain bacteria, some of which are commensals in the gut of animals including man.[10] In recent years, moreover, evidence has appeared indicating that N_2O may be produced in mammalian cells as part of their metabolism.[4,7] When we discovered that N_2O could be involved in neurotransmission in humans,[11–13] we did not realize that we had stumbled on a new biological principle. The generality of our finding was confirmed when nitric oxide (NO), another endogenously produced gas, was also discovered to have a role in neurotransmission.[14,15]

The evidence that N_2O is produced endogenously[4,7] seems to confirm the possibility that it is not only the first gas to have been shown to have a role in neurotransmission but that it is formed by the catabolism of NO.[16,17] This once again highlights the similarities of N_2O and CO_2, which both appear to be endogenously produced as metabolic products in bacterial and mammalian cells. These gases are therefore involved in the physiology of cells from the simplest to the most complex life forms. This is perhaps not surprising, given that N_2O and CO_2 are linked through the nitrogen and carbon cycles (see above), which are crucial to living organisms.

Actions on the nervous system

In higher animals, N_2O and CO_2 have related actions on the nervous system. Both gases in high concentrations cause depression of the central nervous system, leading eventually to coma, respiratory depression and finally death.[18–20] Despite these apparent similarities, however, there are differences in their biological effects.[19,20]

Respiration

The effects of N_2O on respiratory drive are relatively small, with slight depression of the stimulatory response in humans to CO_2 when 50% N_2O in oxygen is breathed.[19] The response to hypoxia is also reduced when 50% N_2O is breathed. Since N_2O at non-anaesthetic concentrations (such as 50%) is an multipotent opioid agonist,[21–23] its depressant effect on respiration could be opioid mediated. Nevertheless, it is much less marked than some other opioids and particularly those that are selective mu-opioid agonists, such as morphine. Competitive opioid antagonists such as

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nalorphine and naloxone can reverse these effects on respiration.[24,25] Such antagonistic outcomes on respiratory depression are extremely rapid as would be expected because they are mediated by direct effects on opioid receptors. Moreover, endogenous and exogenous opioids, including N_2O, inhibit the responses of the brainstem respiratory centre to increased CO_2 blood levels.[19] Thus, the control of respiratory function 'may be directly related to competition for opioid receptor sites by these various gases as well as endogenous opioid agents.'[22] It is therefore significant that the depressant effect of morphine on respiration can be as rapidly reversed by inhalation of 5% CO_2 in oxygen,[26] indicating the possibility that this effect is also receptor mediated.

That this influence of CO_2 is likely to involve receptors is supported by the fact that stimulation of ventilation in man begins within seconds of inhaling low concentrations of the gas, with a maximum response occurring within less than 5 minutes.[20] These stimulatory consequences of CO_2 inhalation disappear rapidly after its withdrawal.[20] Indeed, the CO_2 effect on medullary chemoreceptors and peripheral arterial chemoreceptors are clearly receptor mediated.[20] In these circumstances, it is likely that the effects of CO_2 on the ventilatory neurons in the medullary–pontine region are also mediated by receptors. A further clue that this is an opioid interaction is that, in barbiturate poisoning, which is not mediated by opioid receptors,[27] the administration of CO_2 has no positive effect on stimulating respiration.[26] That various gases influence respiration by interacting directly at opioid receptors is supported by the finding that 100% oxygen altered the *in vitro* binding of ^{3}H-dihydromorphine as well as that of ^{3}H-ketocyclazocine.[28] Dihydromorphine and ketocyclazocine are opioid receptor ligands, so that evidence that *in vitro* binding with these agents is interfered with by oxygen indicates that oxygen itself also binds to opioid receptors and therefore has opioid properties. Other studies have confirmed this work.[31]

Although binding studies with CO_2 using opioid ligands have not been performed, as far as we are aware, the similarity of the actions of the specific opioid antagonistic, naloxone, on CO_2 and N_2O analgesia (see below)[29] may indicate that CO_2 also binds to opioid receptors, to exert some of its respiratory effects.

The pharmacological effects of N_2O are also rapid in onset and offset[19,30] and these consequences are almost certainly receptor mediated[16,17,21,22,28,31–33] and could also be responsible for its actions on the respiratory centres.

Sympathetic activity

Sympathetic activity, which prepares the body for the fright/flight response, is increased by both N_2O[34,35] and CO_2.[20] Nitrous oxide seems to act directly at opioid receptors as well as via the alpha$_2$-adrenergic system.[36] Carbon dioxide potently activates the central adrenergic system[20] and there is some preliminary evidence that it acts directly on mu-opioid receptors,[28] further underlining the similarity between the biological actions of these two gases. The descending adrenergic and nor-adrenergic cell groups form most of the lateral medullary reticular formation and are linked to various vital centres in the medulla oblongata of the brainstem, which includes the respiratory centres.[37]

Apart from the influence of these gases on the adrenergic system, they also have local effects. One which they share is the direct local influence on systemic blood vessels, resulting in vasodilatation.[20,38,39] The results of central sympathetic activation are usually opposite to the local effects of CO_2, whereas the local influence on blood vessels seems to be more important than are the adrenergically mediated vasoconstrictor effects.[20] Nitrous oxide[35,36,38] shares these properties.

Pain perception

Analgesia can be produced by hypercapnia, which triggers the release of endogenous opioids, presumably from the spinal dorsal horn,[29] a region in the spinal cord involved with pain perception. The dorsal horn appears to be implicated because the modification of tail-flick latency persists after spinalization.[29] The authors responsible for these results concluded that hypercapnia may produce direct excitatory effects on spinal or bulbo-spinal enkephalinergic (that is, opioid) interneurones. Since this effect was also present in animals spinalized at lower thoracic levels, at least one population of these neurones must have been in the lumbo-sacral region.[29] Gamble and Milne draw the analogy with N_2O when they state that: 'It is interesting that analgesia induced by the structurally similar compound nitrous oxide can be partly blocked by relatively high doses of naloxone under some circumstances.' They found that analgesia caused by CO_2 was also blocked with relatively high doses of naloxone (2 mg/kg).[29]

Other workers have shown that both N_2O and hyperventilation have similar inhibitory effects on lamina V of the spinal cord, where there is convergence of visceral and high threshold cutaneous pain.[40] These laminae are rich in opioid terminals.[41]

These findings seem to indicate that CO_2, like N_2O, acts directly on mu-opioid receptors, especially in their effects on pain perception and possibly also in their actions on respiration as suggested previously (see above) on the basis of radioreceptor assays.[22]

Other pharmacological actions of CO_2 and N_2O on the central nervous system

Pharmacological, rather than physiological, concentrations of CO_2 produce stress reactions such as anxiety and fear.[42,43] This outcome is probably mediated by stimulation of the sympathetic system[20] and blockade of opioid receptors.[26] In contrast, although also heightening sympathetic stimulation,[19,20] non-anaesthetic doses of N_2O have anti-stress effects[44–47] where the agonistic influence of N_2O on the opioid receptors seems to outweigh its sympathetic activity.[45] Indeed, low doses of N_2O reduce cortisol release.[46]

Both CO_2 and N_2O have antidepressant effects.[48–50] Interestingly, Silver found that such effects of CO_2 were magnified if preceded by a short administration of anaesthetic concentrations of N_2O for 8 or 9 inhalations. Nitrous oxide at 100% used as an anaesthetic, also for a few inhalations (less than a minute's exposure), has an antidepressant action.[49] Furthermore, inhalation of 80% N_2O (with 20% O_2) for between 1.5 and 5 minutes[50] demonstrated that the gas has antidepressant effects. Low concentrations of N_2O mixed with high concentrations of O_2 titrated to a level where the patient is relaxed but fully conscious [so-called psychotropic analgesic nitrous oxide (PAN)][17] also have marked antidepressant properties.[51] Moreover, this work[50,51] indicates that endogenous depressions (which are caused by an underlying biological disturbance) are resistant to the effects of N_2O, whereas reactive depressions (which are related to a stressful life event) are ameliorated by exposure to the gas.

Carbon dioxide at high concentrations (75% in O_2 administered for 30–120 seconds) is effective for treating addicts from whom abused substances such as alcohol[52,53] and opioids[54,55] are suddenly withdrawn.[56] PAN has also been used successfully to treat both acute alcohol[53,57] and opioid[58,59] withdrawal states.

Conclusions

Although we have touched on only some of the properties of these gases, the similar but distinct differences in molecular structure of CO_2 and N_2O seem to be reflected in their similar but contrasting biological actions. It is clear that these gases have overlapping effects. However, CO_2 stimulates respiration whereas N_2O has a slight antagonistic influence on the stimulatory consequences of CO_2. Another distinction is that pharmacological concentrations of CO_2 produce a stress reaction, while the reverse is true of N_2O. Moreover, although both gases can ameliorate withdrawal states after substance abuse, the duration of exposure and the concentrations of the gases required are different.

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1. Laing M. (2003). Some thoughts on the structure and behaviour of N_2O, laughing gas. *S. Afr. J. Sci.* **99**, 109–114.
2. Bowman W.C. and Rand M.J. (1980). *Textbook of Pharmacology,* 2nd edn, pp. 39.14–15. Blackwell, Oxford.
3. Dennis D.T. and Turpin D.H. (Eds)(1990). *Plant Physiology, Biochemistry and Molecular Biology.* Maskew Miller Longman, Singapore.
4. Hyun J., Chaudhuri G. and Fakuto J.M. (1999). The reductive metabolism of nitric oxide in hepatocytes: possible interaction with thiols. *Drug. Metab. Dispos.* **27**, 1005–1009.
5. Lipschultz F., Zafiriou O.C, Wofsy S.C., McElroy M.B., Valois F.W. and Watson S.W. (1981). Production of NO and N_2O by soil nitrifying bacteria. *Nature* **294**, 641–643.
6. Smart R.S. and Bloom A.J. (2001). Wheat leaves emit nitrous oxide during nitrate assimilation. *Proc. Natl Acad. Sci. USA* **98**, 7875–7878.
7. Einarsdottir O. and Caughey W.S.(1988). Interactions of the anesthetic N_2O with bovine heart cytochrome c oxidase. *J. Biol. Chem.* **263**, 9199–9205.
8. Sowa S., Dong A., Roos E.E. and Caughey W.S. (1987). The anesthetic nitrous oxide affects dioxygen utilization by bovine heart and bean seed mitochondrial particles. *Biochem. Biophys. Res. Commun.* **144**, 643–648.
9. Falkowski P.G. (1997). Evolution of the nitrogen cycle and its influence on the biological sequestration of CO_2 in the ocean. *Nature* **387**, 272–275.
10. Trudell J.R. (1985). Metabolism of nitrous oxide. In *Nitrous oxide/N_2O,* ed. E.I. Eger II, pp. 204–210. Elsevier, New York.
11. Gillman M.A., Kok L. and Lichtigfeld F.J. (1980). Paradoxical effect of naloxone on nitrous oxide analgesia in man. *Eur. J. Pharmacol.* **61**, 175–177.
12. Gillman M.A. and Lichtigfeld F.J. (1981). A comparison of the effect of morphine sulphate and nitrous oxide analgesia on chronic pain states in man. *J. Neurol. Sci.* **45**, 41–45.
13. Gillman M.A. and Lichtigfeld F.J. (1981). The similarity of the action of nitrous oxide and morphine. *Pain* **10**, 110.
14. Bredt D.S., Hwang P. M. and Snyder S.H. (1990). Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature* **347**, 768–770.
15. Meller S.T. and Gebhart G.F. (1993). Nitric oxide (NO) and nociceptive processing in the spinal cord. *Pain* **52**, 127–136.
16. Gillman M.A. and Lichtigfeld F.J. (1994). NO comments. *Nature* **367**, 28.
17. Gillman M.A. and Lichtigfeld F.J. (1994). Opioid properties of psychotropic analgesic nitrous oxide (laughing gas). *Perspect. Biol. Med.* **38**, 125–138.
18. Ganong W.F. (1995). In *Review of Medical Physiology,* 17th edn, pp. 625–639. Prentice-Hall, London.
19. Marshall B.E. and Longnecker D.E. (1996). General anesthetics. In *Goodman and Gilman's The Pharmacological Basis of Therapeutics,* 9th edn, ed. J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon and A.G. Gilman, pp. 307–330. McGraw-Hill, New York.
20. Eickenhoff R.G. and Longnecker D.E. (1996). The therapeutic gases. In *Goodman and Gilman's The Pharmacological Basis of Therapeutics,* 9th edn, ed. J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon and A.G. Gilman, pp. 349–359. McGraw-Hill, New York
21. Gillman M.A. (1986). Analgesic (sub-anesthetic) nitrous oxide interacts with the endogenous opioid system: a review of the evidence. *Life Sci.* **39**, 1209–1221.
22. Gillman M.A. (1989). Physico-chemical basis of nitrous oxide activity at opioid receptors. *S. Afr. J. Sci.* **85**, 280–281.
23. Gillman M.A. (1993). Analgesic nitrous oxide should join the opioid family after almost two centuries. *S. Afr. J. Sci.* **89**, 25–24.
24. Alexander F. (1876). In *An introduction to Veterinary Pharmacology,* 3rd edn, pp. 99–122. Churchill Livingstone, Edinburgh.
25. Reisine T. and Pasternak G. (1996). Opioid analgesics and antagonists. In *Goodman and Gilman's The Pharmacological Basis of Therapeutics,* 9th edn, ed. J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon and A.G. Gilman, pp. 521–555. McGraw-Hill, New York.
26. Alexander F. (1976) In *An Introduction to Veterinary Pharmacology,* 3rd edn, pp. 184–190. Churchill Livingstone, Edinburgh.
27. Hobbs W.R., Rall T.W. and Verdoorn T.A. (1996). Hypnotics and sedatives. In *Goodman and Gilman's The Pharmacological Basis of Therapeutics,* 9th edn, ed. J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon and A.G. Gilman, pp. 361–396. McGraw-Hill, New York.
28. Waller D.D. (1985). *Molecular identification of the mode of interactions of nitrous oxide with the opiate receptor system.* M.Sc. thesis, University of the Witwatersrand, Johannesburg.
29. Gamble G.D. and Milne R.J. (1990). Hypercapnia depresses nociception: endogenous opioids implicated. *Brain Res.* **514**, 198–205.
30. Clark M. and Brunick A. (1999). *Handbook of Nitrous Oxide and Oxygen Sedation,* pp. 151–155. Mosby, St Louis.
31. Ori C., Ford-Rice F. and London E.D. (1989). Effects of nitrous oxide and halothane on mu and kappa opioid receptors in guinea-pig brain. *Anesthesiology* **70**, 541–544.
32. Moody E.J., Mattson M., Newman A.H., Rice K.C. and Skolnick P. (1989). Stereospecific reversal of nitrous oxide analgesia by naloxone. *Life Sci.* **44**, 703–709.
33. Daras C., Cantrill R.C. and Gillman M.A. (1983). ^{3}(H)-Naloxone displacement: evidence for nitrous oxide as an opioid agonist. *Eur. J. Pharmacol.* **89**, 177–178.
34. Eisele J.H. (1985). Cardiovascular effects of nitrous oxide analgesia. In *Nitrous Oxide/N_2O,* ed. E.I. Eger II, pp. 126–156. Elsevier, New York.
35. Ebert T.J. and Kampine J.P. (1989). Nitrous oxide augments sympathetic outflow: direct evidence from human peroneal nerve recordings. *Anesth. Analg.* **69**, 444–449.
36. Guo T., Davis M.F., Kingery W.S., Patterson A.J., Limbird L.E. and Maze M. (1999). Nitrous oxide produces antinociceptive response via α_{2B} and/or α_{2C} adrenoceptor subtypes in mice. *Anesthesiology* **90**, 470–76.
37. Williams P.L. (Ed.) (1995). *Gray's Anatomy,* 38th edn, pp. 1076–1077. Churchill Livingstone, New York.
38. Matheny J.L., Westphal K.A., Richardson D.R. and Roth G.I. (1991). Macro- and microvascular effects of nitrous oxide in the rat. *Anesth. Prog.* **38**, 57–64.
39. Roth G.I, Matheny J.L., Falace D.A., O'Reilly J.E. and Norton J.C. (1984). Effect of age on the digit blood flow response to sedative concentrations of nitrous oxide. *Anesth. Prog.* **31**, 17–22.
40. Kitahata L.M, Taub A. and Sato I. (1971). Lamina-specific suppression of dorsal horn unit activity by nitrous oxide and by hyperventilation. *J. Pharmacol. Exp. Ther.* **176**, 101–108.
41. Gillman M.A. and Lichtigfeld F.J. (1985). A pharmacological overview of opioid mechanisms mediating analgesia and hyperalgesia. *Neurolog. Res.* **7**, 106–119.
42. LaVerne A.A. (1953). Rapid coma technique of carbon dioxide inhalation therapy. *Dis. Nerv. Syst.* **14**, 141–44.
43. Scott W., Woods M.D., Chrystal J.H., Cynthia L., D'Amico C.L., Heninger M.D. and Charney M.D. (1988). A review of behavioural and pharmacologic studies relevant to the application of CO_2 as a human model of anxiety. *Psychopharmacol. Bull.* **24**, 149–153.
44. Russel R.W. and Steinberg H. (1955). Effects of nitrous oxide on reactions to 'stress'. *Quat. J. Exp. Psychol.* **7**, 67–73.
45. Gillman M.A. and Katzeff I.E. (1988). Does opioid uncoupling of adrenal responses mediate opioid anti-stress effects. *Med. Sci. Res.* **16**, 207–209.
46. Gillman M.A. and Katzeff I.E. (1989). Antistress hormonal responses of analgesic nitrous oxide. *Int. J. Neurosci.* **49**, 199–202.
47. Clark M. and Brunick A. (2003). *Handbook of Nitrous Oxide and Oxygen Sedation,* pp. 28–32. Mosby, St Louis.
48. Silver G.A. (1953). Carbon dioxide therapy. *Sthn Med. J.* **46**, 283–286.
49. Brill N.Q., Crumpton E., Eiduson S., Grayson H.M., Hellman L.I. and Richards R.A. (1959). Relative effectiveness of various components of electroconvulsive therapy. *Arch Neurol. Psychiat.* **81**, 627–635.
50. Zádor J. (1928). Der Lachgas (N_2O) – Rausch in seiner Bedeutung für Psychiatrie und Neurologie. *Arch. Psychiatrie* **84**, 1–71.
51. Lichtigfeld F.J. and Gillman M.A. (2003). Another marker for depression. *Int. J. Neuropsychopharmacol.* **6**, 91–92.
52. Lichtigfeld F.J. and Gillman M.A. (1982). The treatment of alcoholic withdrawal states with oxygen and nitrous oxide. *S. Afr. Med. J.* **61**, 349–351.
53. Gillman M.A. and Lichtigfeld F.J. (2004). Enlarged double-blind randomised trial of benzodiazepines against psychotropic analgesic nitrous oxide for alcohol withdrawal. *Addict. Behav.* **29**, 1183–1187.
54. Kripke B.J. and Hechtman H.B. (1972). Nitrous oxide for pentazocine addiction and intractable pain. *Anesth. Analg.* **51**, 520–526.
55. Gillman M.A. and Lichtigfeld F.J. (1985). Analgesic nitrous oxide: adjunct to clonidine for opioid withdrawal. *Am. J. Psychiatry* **142**, 784–785.
56. LaVerne A.A. (1973). Carbon dioxide therapy (CDT) of addictions. *Behav. Neuropsychiat.* **4**, 13–32.
57. Lichtigfeld F.J.and Gillman M.A. (1982). The treatment of alcoholic withdrawal states with oxygen and nitrous oxide. *S. Afr. Med. J.* **61**, 349–351.
58. Kripke B.J. and Hechtman H.B. (1972). Nitrous oxide for pentazocine addiction and intractable pain. *Anesth. Analg.* **51**, 520–526.
59. Gillman M.A. and Lichtigfeld F.J. (1985). Analgesic nitrous oxide: adjunct to clonidine for opioid withdrawal. *Am. J. Psychiatry* **142**, 784–785.