Review article
The two faces of Eve: gaseous anaesthesia and inert gas narcosis
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Key words
Anaesthesia, nitrogen narcosis, inert gas narcosis, xenon, pharmacology, physiology, review article

Abstract
Gaseous anaesthesia has been a great boon for medicine. These drugs form a foundation from which modern surgery has sprung, yet their mechanism(s) of actions remains poorly understood. Inert gas narcosis is a limitation of deep sea diving, and its mechanisms also remain poorly understood. In this review article we summarise what is known about the mechanisms of both gaseous anaesthesia and inert gas narcosis, including both lipid-based biophysical models and protein-based biochemical models, as well as explore some striking similarities between the two. These two phenomena may, in reality, be gradations of the same underlying mechanism. Recent findings include biochemical evidence suggesting that both gaseous anaesthesia and inert gas narcosis may be mediated by the occupation of minute spaces within the structure of many biologically important proteins, impairing their ability to undergo conformational changes and biological actions. This is exemplified by exploring the effects of the noble gas xenon, which can behave as either a narcotic gas or gaseous anaesthetic, depending on the partial pressure in which it is present.

Introduction
Volatile gas anaesthesia represents a transformational medical advance of the last 200 years. Introduced 160 years ago, these drugs form a foundation from which modern surgery has sprung. Inhaled anaesthetics have the closest lethal dose to effective dose ratio of all drugs used in medicine, requiring an entire specialty to be developed in order to ensure safe utilization. While gas anaesthesia has been a great boon for medicine, inert gas narcosis has been a limitation of deep sea diving. This limitation has impaired our ability to explore the 70% of our planet covered by water, except through indirect means (i.e., submersibles). In fact, it has been remarked that we know more about the surface of Mars than we do about the deep ocean. A discussion of the seemingly far removed topics of general anaesthesia and nitrogen narcosis leads to many questions. What if gaseous anaesthesia and inert gas narcosis are, in reality, different manifestations of the same phenomenon? More basic is the question: are we adapted to a very narrow ambient pressure environment due to inert gas effects? Previously inert gases have been felt to have no physiologic effects but we herein hypothesize that they have complex, little understood effects that indeed modulate many cell membrane and protein functions. It is through a wider understanding and, perhaps, entertaining the notion that inert gases exert very necessary physiologic ordering effects that we accept as ‘normal’ that we can understand such previously non-investigated phenomena.

Mechanisms
When Behnke et al proposed that nitrogen, or, more broadly, the inert gas fraction of the breathing gas, is responsible for narcosis in 1935, the assertion was based on the Meyer-Overton hypothesis.1 That hypothesis states that the narcotic potency of an anaesthetic (or an inert gas) is related to its lipid solubility.2,3 Lipid solubility is the physical property of inert gases that has been found to correlate most consistently with their narcotic/anaesthetic potency.4 The Meyer-Overton hypothesis, with regards to inert gas narcosis, was found to be tenable by Carpenter when he showed that at iso-narcotic partial pressures (the partial pressure at which each gas shows comparable pharmacologic effects), the inert gas concentration dissolved in the lipid phase is very similar across many gases.5 The partial pressure of various gases required for narcosis varies from 46 kPa to 16.5 MPa (0.045 to 165 Ata).5 Table 1 illustrates the lipid solubility to relative narcotic potency correlation properties (Table 1).4 Once it was established that the site of action was most likely within the lipid phase, hypotheses began to emerge regarding what was actually taking place that would result in narcosis with identical signs and symptoms being induced by a broad collection of gases with no common structural features.4 Several hypotheses have evolved, including hypoxia, depression of metabolism, cell membrane stabilization, membrane stiffening causing decreased ion permeability, inhibition of the sodium extrusion pump, increased production of inhibitory neurotransmitters such as gamma aminobutyric acid (GABA), and interference with adenosine triphosphate (ATP) production.7 These hypotheses fall into two broad categories: biochemical or physical hypotheses. Biochemical hypotheses imply some effect on respiratory enzyme systems, while physical theories imply some interaction with, or within, part of the cell, such as the cell membrane.8 Until recently, no good
evidence had been found to support biochemical changes at pressures relevant to the clinical manifestations of inert gas narcosis. This suggested that the narcotic action is more likely biophysical than pharmacologic, and evolved into the ‘unitary hypothesis of narcosis’: that the mechanism of narcosis is the same for all anaesthetic gases.9

By the late 1950s, the site of action of narcotic gases had been attributed to synapses in the central nervous system. This was deduced largely from the work of Carpenter in the mid 1950s.5 He demonstrated that 31.4−34.5 MPa (310−340 Ata) of argon, a gas with a narcotic potency more than twice that of nitrogen, were required to effect a block of conduction in isolated peripheral nerve preparations, but argon at a mere 1.8 MPa (18 Ata) of pressure was sufficient to abolish any response to electrical stimulus applied to the foot pad of mice.5,10 This suggests strongly that higher level functions in the brain are much more susceptible to inert gas narcosis than peripheral nerves. Later work examining reflex inhibition in the spinal cord demonstrated that inhibitory synaptic mechanisms were affected by inert gas narcosis before excitatory mechanisms, and that inert gas narcosis, like general anaesthetics, affects cells in the anterior horn of the spinal cord.7

LIPID/MEMBRANE HYPOTHESES

Physical hypotheses, based on the polarization and volume of inert gas molecules are simpler and, perhaps therefore, more likely than biochemical hypotheses.11 The critical volume hypothesis of general anaesthesia proposes that narcosis occurs when the anaesthetic agent enters the lipid portion of the cell in sufficient quantity to cause, in particular, the plasma membrane to swell.12 Accordingly, changes in lipid volume ought to differentiate the anaesthetised from the unanaesthetised state. The critical volume hypothesis is supported by observations that anaesthetics and inert gases at increased ambient pressures expand the volume of lipid monolayers and bilayers, bulk phase solvents, oils and even rubber.13 This hypothesis is further supported by the observation that gas anaesthesia can be reversed with the application of hydrostatic pressure (Figure 1).12,14 The quantitative aspect of this hypothesis was developed, which suggested that a 0.4% expansion of the membrane would be required to produce anaesthesia.15

Although elegant in its simplicity, there are critical elements of this hypothesis that do not agree with observations. The critical volume hypothesis predicts that the percentage change in anaesthetic potency ought to be linearly related to pressure, and that the slope of this relationship ought to be the same for all anaesthetic agents.16 This has not been found to be true.17 Quite the opposite, it has been shown that these pressure/anaesthetic interactions are curvilinear, and differ depending on the anaesthetic in question.17 For instance, the amount of nitrogen required to maintain a given level of anaesthesia increases with pressure to approximately 5.1 MPa (50 Ata), at which point it plateaus and there is no further increase for pressures up to about 13.2 MPa (130 Ata).17 Conversely the requirements for isoflurane decrease up to pressures of about 811 kPa (8 Ata), after which they increase sharply, and continue to increase up to pressures of about 10.1 MPa (100 Ata).17 Also, it has been observed that there is no appreciable increase in membrane thickness at narcotic concentrations of various agents.18

Table 1
Narcotic potencies and physical properties of simple gases4,6

<table>
<thead>
<tr>
<th>Gas</th>
<th>Molecular mass (g/mol)</th>
<th>Van der Waals radius (pm)</th>
<th>Anaesthetic pressure (Ata)</th>
<th>Oil-gas partition coefficient (at 37°C)</th>
<th>Relative narcotic potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helium</td>
<td>4</td>
<td>140</td>
<td>190.546</td>
<td>0.016</td>
<td>0.23</td>
</tr>
<tr>
<td>Neon</td>
<td>20</td>
<td>154</td>
<td>87.096</td>
<td>0.019</td>
<td>0.28</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>2</td>
<td>120</td>
<td>138.038</td>
<td>0.05</td>
<td>0.55</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>28</td>
<td>155</td>
<td>33.113</td>
<td>0.069</td>
<td>1.00</td>
</tr>
<tr>
<td>Argon</td>
<td>40</td>
<td>188</td>
<td>15.136</td>
<td>0.13</td>
<td>2.33</td>
</tr>
<tr>
<td>Krypton</td>
<td>83.7</td>
<td>202</td>
<td>4.467</td>
<td>0.4</td>
<td>7.14</td>
</tr>
<tr>
<td>Xenon</td>
<td>131.3</td>
<td>216</td>
<td>0.955</td>
<td>1.8</td>
<td>25.64</td>
</tr>
</tbody>
</table>

Figure 1
The critical volume hypothesis. Molecules of narcotic gases dissolve in the cell membrane and cause it to swell. When the membrane reaches a certain volume narcosis is produced. Pressure reverses this narcotic effect by compressing the membrane.
These observations led to the postulation of the multi-site expansion hypothesis. As stated by Halsey et al, there are five key elements to this hypothesis:

- General anaesthesia or narcosis can be produced by the expansion of more than one molecular site and these sites may have different physical properties.
- The physical properties of a molecular site may themselves be influenced by the presence of anaesthetics or pressure (i.e., compressibility).
- The molecular sites do not behave as if they were bulk solvents but have a finite size and a finite degree of occupancy.
- Pressure need not necessarily act at the same site as the anaesthetic in order to reverse anaesthesia. Depending on the anaesthetic, one of the sites may predominate in determining the interaction between anaesthesia and pressure.
- The molecular sites for anaesthesia are not perturbed by a decrease in temperature in a manner analogous to an increase in pressure.

Other work on membrane model systems suggested that anaesthetic gases may also alter the permeability of ions. While examining cation permeability in membrane model systems, it was observed that n-alkyl alcohols, chloroform and ether result in a transient, reversible increase in membrane cation permeability. Inert gases behave the same way, as demonstrated by in vivo examination of cerebrospinal fluid (CSF) levels of sodium, potassium and chloride while measuring auditory evoked potentials in cats. A significant decrease in CSF sodium and chloride was found, as well as the amplitude of cortical auditory evoked potentials in animals compressed to 1.1 MPa (11 Ata) breathing a mix of 80% nitrogen/20% oxygen or 80% argon/20% oxygen when compared to breathing 80% helium/20% oxygen at 1.1 MPa (11 Ata) or room air at ambient pressure.

This is further supported by studies by Johnson and Miller as well as by Galey and van Nice. In liposomes exposed to butanol, ether or nitrogen in doses that would be just sufficient to abolish the righting reflex in newts, an increase in the permeability for potassium and rubidium was observed, and application of 15.4 MPa (152 Ata) of pressure was required to counterbalance the permeability changes. Pressures of nitrogen up to 8.9 MPa (88 Ata) stimulate active sodium efflux and potassium influx across the red blood cell membrane, and the effect is abolished by ouabain. In addition, hyperbaric pressures of the non-narcotic gas helium, rather than nitrogen, tended to inhibit active sodium and potassium transport. Other work by this group using rat brain synaptosomes showed that hyperbaric pressures of argon would stimulate potassium uptake by the synaptosomes while 7.0 MPa (69 Ata) of helium or hydrostatic pressure inhibited the accumulation of potassium. This suggests that anaesthesia and narcosis may be the downstream product of gases dissolving into the membrane at various sites, altering ion conduction, and thus synaptic conduction, and ultimately consciousness.

More recently, Abraini has modified this multi-site expansion model based on results obtained from human trials using hydrogen, a relatively non-narcotic gas, as the diluent gas. He has proposed theoretical reconsideration of the interaction between inert gases at hyperbaric pressure, and the effects of pressure itself. According to this hypothesis, narcotic gas and pressure act at different hydrophobic sites and narcosis occurs when a critical level of expansion is reached at some cellular hydrophobic site in the central nervous system. With respect to light inert gases, all narcotic gases act at a common hydrophobic region through a non-specific mechanism.

This hypothesis suggests that the psychotic-like symptoms observed in humans at high pressure may be a paroxysmal symptom of narcosis, not simply a manifestation of the high pressure nervous syndrome (HPNS), and are a result of the sum of the individual narcotic potencies of the various inert gases in the breathing mix. This was tested mathematically against various lipid solubility theories of inert gas narcosis and was found to be sound. This suggests that, depending on the environmental parameters (breathing mix, pressure), symptoms of inert gas narcosis or HPNS appear when a critical imbalance is reached between the narcotic actions of inert gas and the actions of pressure, which tend to reverse narcosis, on their respective hydrophobic sites. Accordingly, inert gas narcosis and HPNS can antagonise each other, or can occur simultaneously.

Given the understanding that inert gas narcosis was somehow connected to changes in synaptic conduction in the central nervous system, some researchers began to investigate changes in neurotransmitters with hyperbaric exposure. Changes in levels of dopamine and norepinephrine have been observed by several groups, but whether an increase or decrease is observed seems to depend on what area of the brain is under investigation rather than the pressure applied. For instance, dopamine and norepinephrine were shown to be decreased in response to 10.1 MPa (100 Ata) trimix (helium/nitrogen/oxygen) and to 2.0 MPa (20 Ata) nitrogen/oxygen mixtures in the hypothalamus, but were increased in the caudate nucleus.

Unfortunately these changes may not have anything to do with inert gas narcosis. Rostain and Forni were able to demonstrate a similar increase in striatal dopamine release in response to 9.1 MPa (90 Ata) helium/oxygen mixture, 9.1 MPa (90 Ata) helium/nitrogen/oxygen mixture (5% nitrogen), and 9.1 MPa (90 Ata) helium/hydrogen/oxygen mixture (66% hydrogen). These mixes should have quite different narcotic potencies, but appeared to cause the same change in dopamine levels. These changes were attributed to, and are likely the result of, pressure alone, not narcosis. Balon et al, while also looking at striatal dopamine release, found a 20% decrease in rats exposed to 3.0 MPa (30 Ata) breathing a nitrogen/oxygen mix.

This suggests that neurotransmitter release in response
to hyperbaric exposure and inert gases is quite complex. Exposure to low pressures, breathing a mixture of nitrogen/oxygen, appears to result in a decrease in striatal dopamine release, while exposure to high pressures, breathing a helium/oxygen mixture, increases dopamine release. Unfortunately it is very difficult to separate out the effects of the inert gas from the effects of pressure per se because gases with high narcotic potency will result in unconsciousness at high pressures. Thus, in order to expose an in vivo preparation to high pressures, helium must be used as a diluent gas if any sort of behavioural observations are to be made. So long as gas pressure is used to generate a hyperbaric exposure, it will remain near impossible to distinguish the effects of high-pressure helium from those of pressure per se. Nonetheless, it would appear that there are consistent, reproducible changes in dopamine release in response to hyperbaric exposure. This suggests that neurotransmission is likely altered under hyperbaric conditions, and may offer a partial explanation of inert gas narcosis.

Other work has suggested that nitric oxide may play a role in narcosis. Vjotosh et al found that when rats were compressed to 4.2 MPa (41 Ata) breathing air, they showed alterations in motor activity at 0.5–1.2 MPa (5–12 Ata), ataxia at 1.0–3.4 MPa (10–34 Ata), and side body position at 2.6–4.2 MPa (26–41 Ata). These were taken as signs of nitrogen narcosis. When treated with the nitric oxide synthase inhibitors L-NAME or 7-NI, the above mentioned signs were abolished or attenuated. While interesting, these results must be taken with a grain of salt. Air breathing at pressures greater than 1.0 MPa (10 Ata) makes acute oxygen toxicity a serious risk. Since seizures are one of the symptoms of acute oxygen toxicity the indicators this group used as signs of narcosis may make narcosis and oxygen toxicity difficult to distinguish.

PROTEIN/METABOLIC HYPOTHESES

Research attention is now focusing on the possibility of direct interactions between inert/anaesthetic gases and proteins, lipoproteins, and other hydrophobic sites within the cell. Much of this evidence comes from the anaesthesia community and the study of volatile, inhaled anaesthetics in general. If inert gas narcosis was solely a matter of gas dissolving in lipid membranes, it would be expected that the onset of narcotic effects would be linearly related to the rate of increase in pressure. That this relationship is, in fact, sigmoidal suggests that the inert gas molecules are interacting with protein receptors directly and act as allosteric modulators.

The idea that the mechanism underlying anaesthesia involves an interaction with proteins is not new. This was first proposed in 1875 by Claude Bernard. He based this conclusion on the way some anaesthetic potencies deviated from that which would be predicted from their lipid solubility alone, combined with the understanding that many proteins contain small hydrophobic domains that would allow for interactions with small, hydrophobic compounds. Unfortunately the interactions between proteins and narcotic compounds appear to be very short-lived (a millisecond or less). Conventional binding assays are simply unable to measure such low-affinity binding.

Since direct measurements of binding are not possible, those interested in studying protein-based mechanisms of anaesthesia and narcosis have resorted to molecular pharmacology and assays of protein activity in various in vitro preparations. These techniques have evaluated possible protein/anaesthetic interactions based on two criteria: ‘plausibility’ and ‘sensitivity’. Plausibility refers to the degree to which changes in protein activity observed in the preparation line up with our preconceptions of anaesthetic mechanisms. For instance, it is believed that anaesthesia and narcosis are products of CNS depression; therefore, the observation that an anaesthetic inhibits proteins involved in excitatory synaptic transmission, or activates proteins involved in inhibitory synaptic transmission would fit the ‘plausibility’ criterion. The sensitivity criterion would come into play in order to evaluate the dose-dependence of the observed changes in protein activity. This criterion would be satisfied if the observed in vitro EC50 (effective concentration for 50% of the effect) were similar to the observed clinical EC50. Plausibility, in this sense, is certainly a very fuzzy concept. Our understanding of the neurophysiology of consciousness is very limited. Consequently our understanding of altered states of consciousness is even more limited, so plausibility is very much open to the subjective interpretation of the investigator.

Inhalational anaesthetics including inert gases have been investigated in several different in vitro systems, and have been found to alter the functions of many enzymes, receptors, transporters, ligand- and voltage-gated ion channels as well as structural proteins. A growing body of evidence suggests that inhalational general anaesthetics work through interactions with proteins, particularly post-synaptic ligand-gated ion channels. These interactions fit well, not only with the plausibility criterion, but with the sensitivity criterion; the doses observed to have appropriate in vitro effects are very similar to clinical doses used to produce general anaesthesia.

The idea that inhaled anaesthetics exert their effects through modulation of inhibitory post-synaptic ligand-gated ion channels is interesting. When activated by the binding of the appropriate ligand (e.g., gamma aminobutyric acid, GABA) these chloride channels open and chloride flows into the cell causing a hyperpolarisation and decreasing the likelihood of action potential propagation. Clinically effective concentrations of several inhaled anaesthetics, as well as high partial pressures of nitrogen, have been demonstrated to potentiate both GABA- and glycine-modulated chloride currents in vitro. Induction of the GABA receptor system is not the only ligand-gated ion channel that anaesthetic and narcotic gases have been demonstrated to interact with,
and alter the function of. Specifically, it has been elegantly demonstrated by Balon et al that the inhaled anaesthetic nitrous oxide exerts its anaesthetic effects via inhibition of the NMDA receptor.\(^{36}\)

Several problems still exist with the hypothesis that inhaled anaesthetics operate through the modulation of ligand-gated ion channels such as the GABA receptor. First, \textit{in vitro}, it has been shown that at high doses of anaesthetic drugs (above 1 mM), GABA\(_\text{A}\) activity tends to be inhibited, yet clinically, increasing anaesthetic doses lead to deeper anaesthesia, not reversal.\(^{35}\) Second, in neonatal rodents, chloride gradients are reversed, thus GABA acts as an excitatory neurotransmitter, but inhaled anaesthetics are still effective in these animals, although slightly higher doses are required.\(^{37}\) Third, if the anxiolytic effect of benzodiazepines is a result of potentiation of the GABA\(_\text{A}\) receptor, inhaled anaesthetics must act through a different mechanism since their effect is decidedly non-anxiolytic.\(^{37}\) The early stages of general anaesthesia induced with inhaled anaesthetics produces an excitatory phase, which can produce seizure-like activity, whereas benzodiazepines prevent seizures. Fourth, chloride channel blockers and GABA\(_\text{A}\) antagonists have only minimal effects on the potency of inhaled anaesthetics.\(^{38,39}\) This evidence suggests that, although volatile anaesthetics can modulate ligand-gated chloride channel activity at clinically relevant concentrations \textit{in vitro} (and possibly \textit{in vivo}), it is unclear how this effect is related to anaesthesia.

Anaesthesia may be a product not of interaction with a single protein, but of interaction with multiple molecular targets. This is suggested by the observations that inhaled anaesthetic agents affect multiple proteins, as well as the fact that multiple anaesthetics with diverse molecular structures all produce the same end result: anaesthesia.\(^{40}\) There is evidence for unique binding sites for several inhaled anaesthetics on a single target, but it seems unlikely that a single molecular target would have specific binding sites for diverse molecular structures ranging from xenon to nitrous oxide to sevoflurane.\(^{41}\) Other evidence suggests that there may be a single, selective target for each anaesthetic, but it seems unlikely that all these molecular targets would lead to the same end result of anaesthesia.\(^{42}\) However, this finding does provide reasonable grounds to believe that interactions with multiple molecular targets may converge to produce the single effect of anaesthesia.\(^{40}\)

It should be remembered that specific interaction with a protein target and multiple sites of action are not mutually exclusive. It is well understood that adenosine triphosphate (ATP), oxygen and calcium all bind selectively with multiple different targets. Volatile anaesthetics have also been shown to bind selectively with multiple different targets, including firefly luciferase, serum albumin, myoglobin, adenylate kinase, haloalkane dehalogenase and T4 lysozyme.\(^{33,44}\) Even more compelling evidence comes from autoradiograms of rat brain slices probed with a radiolabelled halothane derivative. The radiographs revealed widespread binding of the labelled halothane derivative throughout the brain. The distribution of the labelled halothane derivative did not match that of any known receptor or channel (Figure 2).\(^{45}\) Furthermore, the binding was reduced to background levels in the presence of a 10-fold excess of unlabelled halothane. When extracted and separated, multiple brain proteins were found to be specifically labelled in a saturable and stoichiometric manner with estimated affinities near the

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**Figure 2**

Autoradiogram of rat brain section photoaffinity-labelled with radioactive halothane. Degree of halothane binding is indicated by level of darkness; no other staining has been applied to the section. The binding shows little regional preference and is reduced to near-background levels in the presence of a tenfold excess of unlabeled halothane; labels indicate various brain regions.\(^{45}\) (with permission)
clinical EC\textsubscript{50}.\textsuperscript{40} The dramatic inhibition of labelling by non-radioactive halothane indicates that most halothane binding is saturable and specific, showing that many proteins could be involved in anaesthetic action.\textsuperscript{45}

The nature of the interaction between anaesthetics and proteins may lie in the structure of proteins themselves. It is understood that proteins fold into complex 3-dimensional structures that are not solid, but rather contain cavities. These cavities are believed to be critical structural elements in protein function as they introduce areas of instability that allow conformational changes to take place.\textsuperscript{46} These cavities within the hydrophobic core of proteins provide plausible binding sites consistent with the observation that anaesthetic potency is correlated with lipid solubility (Figure 3). That potent anaesthetics exhibit weak polarity is also consistent with the hypothesis that they bind in protein cavities, as most cavities are also weakly polar.\textsuperscript{46,47} The elements of protein secondary structure that form the surface of these cavities could also provide an explanation for the weak stereo selectivity observed with some anaesthetics such as isoflurane.\textsuperscript{48} Occupancy of these cavities by anaesthetic molecules could affect anaesthesia by limiting the motion that underlies protein activity.\textsuperscript{47,49} Studies have indicated that occupancy of cavities by small, hydrophobic molecules does reduce protein motion.\textsuperscript{46} This is clearly a multiple-target hypothesis, and nicely reconciles results from binding and functional studies.

**Xenon**

Thus far, the terms ‘inert gas narcosis’ and ‘anaesthesia’ have been, to a degree, used interchangeably. This is based on the hypothesis that all anaesthetics act through the same basic mechanisms, and the observations that inert gas narcosis resembles early stages of anaesthesia and that inert gases can produce general anaesthesia if delivered at sufficiently high partial pressures. The inert gas xenon exemplifies much of this overlap. Xenon is an inert gas with a narcotic potency sufficiently high that it can be used as a general anaesthetic at 101.3 KPa (1 bar).\textsuperscript{50} If it can be assumed that inert gas narcosis exists as a single condition regardless of the inert gas in question (to a large extent the research community has already done so in order to try to separate narcotic effects from pressure effects), what is understood about the mechanism of xenon anaesthesia can be extended to all inert gas narcosis and gaseous anaesthesia.

Investigations into the mechanisms of action of xenon gas anaesthesia present a microcosm of investigations into the mechanism of action of the entire scope of inhalational anaesthesia. Hypotheses abound regarding both lipid (membrane) and direct interaction with protein. In support of the membrane hypothesis, there is evidence that xenon can interact with lipid bilayers and change surface tension, bilayer volume, and pressure within the bilayer.\textsuperscript{51} There is also evidence to suggest that the xenon dissolved in the membrane, despite its high lipid solubility, may not reside within the central core, or tail region of the membrane, but may preferentially migrate to the amphiphilic region around the head groups after dissolving in the membrane.\textsuperscript{52} This accumulation of xenon molecules in the membrane is believed to affect the structure and function of proteins embedded within the membrane.\textsuperscript{53} In particular, it is believed that changes in surface tension and pressure within the membrane result in conformational changes in ion channels within the synaptic terminals of neurons, resulting in decreased conduction leading to anaesthesia.\textsuperscript{52}

The greatest criticism levelled against much of the work done on the basis of the lipid hypothesis of narcosis, be that in vivo or in silico, is the fact that most of these studies are done using dipalmitoylphosphatidylcholine (DPPC) bilayers as a membrane system.\textsuperscript{32} DPPC is an easily obtained egg yolk phospholipid that contains no double bonds to oxidise and readily forms into a biologically relevant liquid crystalline bilayer. Unfortunately it is also far too simple a system to accurately model a biological membrane. Living plasma membranes, especially those of neurons, contain a highly heterogeneous mixture of lipids, some saturated, some unsaturated, some charged, some neutral, as well as cholesterol.\textsuperscript{53} Biological membranes also contain various proteins, which make up approximately 50% of the mass of the membrane.\textsuperscript{54} In addition, it is now understood that the inner and outer leaflets of biological membranes are composed of different phospholipids, and that the membrane

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**Figure 3**

Anaesthetic gases may exert their effects through the occupation of small cavities in proteins. A depicts a protein in its resting, inactive state. When activated (B), the protein undergoes a conformational change. C depicts a protein in its inactive state with molecules of an anaesthetic or narcotic gas occupying cavities within the tertiary structure of the protein. In D, the protein is unable to undergo conformational change, and thus remains inactive.
is arranged laterally into distinct areas known as lipid rafts, which are composed of distinct lipids and proteins. For example, the nicotinic acetylcholine receptors in nerve and muscle cells require the presence of cholesterol and anionic phospholipids in their immediate vicinity in order to function properly. The simple membrane systems often used experimentally cannot approximate well the behaviour of biological membranes and thus their findings for the mechanism of narcosis are questionable. On the other hand, these models do demonstrate that it is plausible that the membrane is, at least in part, responsible for the mechanism of narcosis.

There is evidence that xenon anaesthesia/narcosis may be a product of interaction with proteins. Whereas most general anaesthetics have been shown to enhance the inhibitory activity of GABA receptors, xenon seems to have little or no effect on them. Instead, it appears that xenon inhibits the excitatory action of the N-methyl-D-aspartate (NMDA) receptor and, to lesser extents, the neuronal nicotinic receptor. A single xenon molecule was found to bind to a wide range of soluble and membrane-bound proteins, such as the NMDA receptor, are difficult to crystallise, so xenon has not been crystallised with the protein actually believed to be its target, but with soluble surrogate proteins. Xenon has been crystallized with urate oxidase, a prototype of various intracellular globular proteins, and with annexin V, a protein with structural and functional characteristics that allow it to be considered a prototype of the NMDA receptor. A single xenon molecule was found to bind to both these proteins in a flexible, hydrophobic cavity within the structure of the protein. This is consistent with both the hydrophobicity of xenon, and with the previous hypothesis that anaesthetic molecules exert their effect by binding proteins in these hydrophobic cavities. This suggests that it is plausible that xenon binds with the NMDA receptor, but that it is also capable of binding to a wide range of soluble intracellular proteins, consistent with the hypothesis that anaesthesia/narcosis is likely the ultimate product of multiple drug/protein interactions.

Other anaesthetic compounds that are believed to operate primarily by inhibiting NMDA-receptor signalling, such as nitrous oxide and ketamine, have been observed to increase both global and regional cerebral metabolism in humans. Contrary to expectations, xenon anaesthesia depressed cerebral metabolism both globally and in multiple regions of the brain. This suggests strongly that the mechanism of xenon anaesthesia/narcosis is not simply the inhibition of NMDA receptors.

**Conclusion**

Inert gases clearly have physiological effects. The fact that the inert gas xenon fills voids in proteins is itself provocative. Work has shown inert gases act on neurons, nerve conduction and consciousness. Today, a picture of how inert gases function as anaesthetics is beginning to emerge. The idea that inert gas narcosis can be reduced to a single cause is likely incorrect. The symptoms displayed vary widely, not only between different individuals, but also between different exposures for the same individual. The conditions required to bring about the onset of inert gas narcosis (ambient pressure, gas mix being breathed, temperature, psychological factors) also appear to vary widely. Hypotheses focusing exclusively on either cell membranes or protein interactions do not appear to tell the whole story. At this point in the research it would appear that there are elements of truth in both of these theories. Continued research into both inert gas narcosis, and the mechanisms of general anaesthesia, particularly mechanisms pertaining to inhaled anaesthetics, is likely to further understanding of both conditions. The time may be nearing when, in order to truly understand the mechanisms of inert gas narcosis and general anaesthesia, hypotheses will need to be able to bring together understanding gained from both lipid- and protein-based models in order to construct a single model that can explain all the observations.

It is clear that pressure, combined with inert gases, changes cell functions. From such an observation, a series of conclusions tumble: membranes, proteins and other bioactive molecules have evolved to their functions on Earth’s surface perhaps in a selected ‘pressure/inert gas window of normal activity’. Therefore, inert gases may not be truly ‘inert’, as they exert ordering effects upon membranes, proteins and cell signalling. They clearly do not react by ionic bonding or undergo metabolism like oxygen and carbon dioxide but neither are they non-participators, the inert gases provide order. The proposed mechanisms discussed in this article are summarised briefly in the Appendix.

For readers who would like further background information on inert gas narcosis or mechanisms of anaesthesia, please refer to the chapter by Bennett and Rostain on inert gas narcosis in *Bennett and Elliott’s physiology and medicine of diving*, or, for mechanisms of anaesthesia, a 2001 article by RG Eckenhoff in *Molecular interventions*.
### Appendix

Summary table of the major hypotheses as to the mechanism(s) of gas anaesthesia and inert gas narcosis

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<th>Mechanism of anaesthesia</th>
<th>Evidence</th>
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<td><strong>Physical models</strong></td>
<td></td>
<td></td>
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<tr>
<td>Critical volume</td>
<td>Physical expansion of plasma membrane</td>
<td>Increasing partial pressures of anaesthetic gases increase the volume of various lipids. Increasing pressure can reverse anaesthesia.</td>
</tr>
<tr>
<td>Multi-site expansion</td>
<td>Physical expansion of discrete sites in the plasma membrane</td>
<td>Slope of anaesthetic potency vs. pressure found to be non-linear and different for various agents.</td>
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<tr>
<td>Changes in ion permeabilities</td>
<td>Anaesthetic agents alter plasma membrane permeabilities</td>
<td>Application of inert and anaesthetic gases alters ion content on either side of various lipid membranes.</td>
</tr>
<tr>
<td>Modified multi-site expansion</td>
<td>Anaesthetic gases and pressure have separate actions at</td>
<td>All light anaesthetic gases interact with the membrane at a common hydrophobic site. Narcosis and HPNS can coexist in the same individual at</td>
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<td>separate membrane sites</td>
<td>the same time.</td>
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<td><strong>Biochemical models</strong></td>
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<td>Altered synaptic conduction</td>
<td>Changes in neurotransmitter release at CNS synapses affect</td>
<td>Hyperbaric exposure and breathing gas mix alter the release of various CNS neurotransmitters.</td>
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<td>consciousness</td>
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<td>Single-protein interactions</td>
<td>Anaesthesia results from the interaction of anaesthetic</td>
<td>Various anaesthetics alter conduction through specific post-synaptic ion channels.</td>
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<td>molecules with certain specific functional proteins</td>
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<tr>
<td>Multi-protein interactions</td>
<td>Anaesthesia results from the specific interaction of anaesthetic molecules with multiple functional proteins</td>
<td>Many anaesthetic gases have been found to bind selectively with multiple different target proteins. Halothane binds in a competitive manner to multiple proteins throughout the brain. Protein tertiary structures contain small hydrophobic cavities with weak polarity. Occupation of these cavities by small molecules reduces protein motion.</td>
</tr>
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</table>

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#### References

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34 Cantor RS. The lateral pressure profile in membranes: A physical mechanism of general anesthesia. Biochemistry. 1997;36(9):2339-44.


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