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**ABSTRACT**

This mini-review summarizes current ideas of how hyperbaric gases (>1-10 ATA) affect neuronal mechanisms of excitability through molecular interaction with membrane components. The dynamic nature of the lipid bilayer, its resident proteins, and the underlying cytoskeleton, makes each respective nanostructure a potential target for modulation by hyperbaric gases. Depending on the composition of the gas mixture, the relative concentrations of O<sub>2</sub> and inert gas, and total barometric pressure, the net effect of a particular gas on the cell membrane will be determined by the gas' i) lipid solubility, ii) ability to oxidize lipids and proteins (O<sub>2</sub>), and iii) capacity, in the compressed state, to generate localized shear and strain forces between various nanostructures. A change in the properties of any one membrane component is anticipated to change conductance of membrane-spanning ion channels and thus neuronal function.

## INTRODUCTION

The range of hyperbaric pressure that humans can survive, without protection from a sealed 1 atmosphere pressure suit or submersible, extends from just beneath sea level (1 atmosphere absolute, ATA<sup>1</sup>) down to a maximum pressure of ~70 ATA,<sup>2</sup> which is equivalent to ~2,300 feet of sea water (fsw) (26, 30). The caveat, of course, is that the aquanaut descending over this continuum of increasing ambient pressure must use specialized breathing equipment that delivers gas to their lungs at a pressure equivalent to ambient pressure. The *level of inspired O<sub>2</sub> and the mixture of balance gases* have to be selected carefully for the desired depth to avoid the powerful, wide ranging but harmful effects on neurological function of breathing hyperbaric oxygen (HBO<sub>2</sub>) and hyperbaric nitrogen (HBN<sub>2</sub>). This means *decreasing* the fractional concentration of N<sub>2</sub> in air to less than 0.79 to avert the euphoric irrationality of inert gas narcosis (IGN), otherwise known as nitrogen narcosis, and carefully regulating inspired O<sub>2</sub> to avoid the violent, uncontrollable seizures of central nervous system (CNS) oxygen toxicity; reviewed in (30). At even greater depths, in the absence of CNS oxygen toxicity and IGN, diver performance can still be impaired by a constellation of debilitating symptoms known collectively as high pressure nervous syndrome (HPNS), which includes, but is not limited to, muscular tremors, loss of coordination and memory deficits (30, 84).

In each case, hyperbaria is the requisite condition for induction and maintenance of neurological dysfunction. The diversity of molecular and cellular mechanisms

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<sup>1</sup> 1 atmosphere absolute (ATA) is barometric pressure (P<sub>B</sub>) at sea level. 1 ATA = 14.7 psig = 760 mm Hg = 0.1 MPa.

<sup>2</sup> Ambient pressure increases 1 atmosphere every 33 feet of sea water (fsw), where 33 fsw = 2 ATA (1 ATA air + 1 ATA water), 66 fsw = 3 ATA (1 ATA air + 2 ATA water), *etc.*

responsible for each neurological condition is readily apparent when the suspected underlying causes are considered: CNS oxygen toxicity is attributed to the harmful effects of various species of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (3, 30); IGN is attributed to the narcotic action of high-pressure nitrogen (30, 102); and HPNS is attributed to the direct effects of hydrostatic compression of the CNS in the absence of narcosis (30, 84). While the primary stimuli and mechanisms appear to be vastly different in each situation, *the chief cellular targets, generally speaking, are anticipated to be the same at the nanostructural level*: a cytoskeleton tethered to an overlying plasma membrane containing membrane-spanning proteins that comprise ion channels, membrane transporters and receptors (47, 54, 78, 95).

The first goal of this mini-review is to summarize current ideas of how gases breathed over the *physiological range of tolerable pressures* affect the components of neuronal membranes. The second goal is to consider how these changes, in turn, may modulate neuronal mechanisms of excitability. First, we will briefly review the fundamental components of the cell membrane and their dynamic nature as an introduction to the potential membrane targets of hyperbaric gases. Next, we will define the environmental conditions that produce oxygen toxicity and IGN and review the effects of HBO<sub>2</sub>, inert gases, and pressure per se on cell membranes. The topic of pressure sensitivity (barosensitivity) will be limited to barometric pressures ( $P_B$ ) <10 ATA and, thus, will not include HPNS (30, 84).

Our final goal is to emphasize areas of hyperbaric membrane research requiring additional study. There is relatively little known about how hyperbaric gases and pressure (<10 ATA) affect cell membranes. Several hypotheses and theories have been

proposed to explain the membrane mechanisms for oxygen toxicity, IGN, barosensitivity and pressure reversal of IGN, but comparatively few experimental studies have actually studied these mechanisms. This is in contrast to the vast literature on cellular mechanisms of gas sensitivity of the CNS at normobaric pressure; e.g. (1, 68, 98). There are two main reasons for this deficiency. First, the technology and tools needed for hyperbaric membrane research are not readily available and must be developed, which is no simple task (28, 29). To date, this has been successfully accomplished for electrophysiology (24, 28, 85), amperometry (45, 61), fluorescence microscopy (38, 102) and, most recently, atomic force microscopy (AFM) (20).

Second, of the hyperbaric studies (*in vitro*) to date, relatively few have focused on the physiological and biophysical effects of small to moderate levels of hyperbaria (<10 ATA). These levels of pressure are relevant for most of the commonly encountered problems in diving and hyperbaric medicine (30).<sup>3</sup> Previous studies have tended to focus on the effects of extreme hyperbaria, usually  $\geq 100$  ATA, which is well beyond the level of pressure that any terrestrial mammal ever experiences, and are discussed elsewhere (26, 30, 51). The neurological disorders we address here are all initiated at  $P_B \sim 3-4$  ATA and do not need exceed 10 ATA (26, 30, 50-52).

## **THE NEURONAL MEMBRANE**

Neuronal membranes are similar in structure and function to all eukaryotic cells and consist of a lipid bilayer tethered to the underlying cytoskeleton, with proteins embedded or loosely attached to the bilayer (47, 54, 78, 95). A significant change in any

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<sup>3</sup> The exception to this statement is HPNS, which is initiated at 10-15 ATA and can be a problem with deeper technical dives using heliox or hydroliox gas mixtures (30).

one of these structural components is likely to impact the configuration of functional proteins (ion channels, transporters and receptors). Each nanostructure is potentially vulnerable to physical reconfiguration by increased gas pressure due to the gas' i) lipid solubility (8, 102), ii) ability to oxidize lipids and proteins ( $O_2$ ) (3, 33, 88), and iii) capacity through compression to generate localized shear and strain forces between membrane-bound proteins and lipids with differential compressibility (50, 52).

The lipid bilayer consists of phospholipids, cholesterol, integral proteins, peripheral proteins, glycoproteins, glycolipids and lipoproteins in different ratios. The composition of polyunsaturated fatty acids (PUFAs) and temperature determine the membrane fluidity, which under normal conditions has the viscosity of light oil (41). For example, the *cis*-double bonds of PUFAs produce kinks in the hydrocarbon chains and make them more difficult to pack together, enabling the cell membrane to remain fluid (80), which enables rapid lateral diffusion of lipid and protein molecules (41, 94). Abnormal membrane function, conversely, is associated with decreased membrane fluidity. For example, membrane fluidity is decreased by lipid peroxidation of PUFAs (93) and phase transition from reduction in temperature (43).

Phospholipids are amphipathic lipids, having their hydrophobic lipid tails oriented inwards and the hydrophilic phosphate groups facing outwards towards the cytosol and extracellular compartment, which gives them their unique functional properties. Phospholipids spontaneously aggregate into bilayers; when torn apart, phospholipids reassemble rapidly into a bilayer configuration. Cell membranes are relatively impermeable to water-soluble molecules; however, gases of moderate-to-high lipid solubility are freely permeable and can impart neuromodulatory changes, such as IGN, at

hyperbaric pressure (see below). The thickness of a bilayer is approximately 5-7 nm (50-70 Å), and barely discernible with a transmission electron microscope (46); however, its surface (21) and biophysical properties can be readily explored using an AFM (75). The two-dimensional fluidity of the lipid bilayer causes thermal fluctuations (i.e., flickering) or “Brownian motion.” Membrane fluidity is reduced by cholesterol, which provides structural stability to the phospholipid molecules and enhances the barrier function to reduce permeability.

Membrane-associated proteins serve various functions, including tethering the cytoskeleton to the extracellular matrix or adjacent cell (13). Membrane-cytoskeleton adhesion not only stabilizes the cell membrane, but also assists cell functions and influences viability. Cytoskeletal rearrangements are triggered by changes in intracellular  $\text{Ca}^{2+}$  and associated downstream signaling pathways, and are critical for many functions, including neuronal growth cone extension and synaptic plasticity (39). Extensive membrane blebbing and defective locomotion occur in cells lacking membrane tethering protein Filamin A (18), which demonstrates the importance of membrane-cytoskeleton bonds.

Intrinsic or integral proteins are embedded in the lipid bilayer and function to move ions or small molecules in and out of the cell or act as receptors. Protein channels have varying degrees of selectivity and permeability for ions and small molecules, including gases. In this last context, exciting research supports the novel idea that aquaporins—water channels—also function as gas channels, enabling rapid transmembrane movement of  $\text{CO}_2$  (17). Presumably, other gases can also pass through these gas channels; however, this has not been tested experimentally. Some ion channels

are mechanosensitive, responding to expansion and contraction of the cell membrane (60). Thus, under conditions of abnormally high bilayer tension, mechanosensitive ion channels (unresponsive under normal conditions) are progressively activated (i.e. mechanoresponsive) via shear forces through perturbed membrane-cytoskeletal interactions. Extrinsic or peripheral proteins (*e.g.* phospholipase enzymes, sphingomyelinase, etc.) are loosely bound to the membrane surface and serve many functions, including receptor-mediated signal transduction. In this regard, imbalances in synaptic neurotransmission, and thus postsynaptic receptor function have been identified as key components for the hyperexcitability observed in barosensitivity (84).

To summarize, cell membranes are dynamic, fluid structures that are intimately linked to neuronal function, including ionic mechanisms of excitability. The dynamic nature of the lipid bilayer, its resident proteins and underlying cytoskeleton makes each respective nanostructure a potential target of molecular O<sub>2</sub>, molecular N<sub>2</sub>, reactive species and physical pressure under hyperbaric conditions as dictated by the composition of breathing gas.

## **HYPERBARIC OXYGEN TOXICITY**

*Oxygen toxicity occurs under what conditions?* CNS oxygen toxicity occurs when breathing pure O<sub>2</sub> at greater than 2-3 ATA. Oxygen toxicity presents as grand mal convulsions with little to no warning. Sometimes seizures are preceded by various autonomic, motor and cardiorespiratory signs and symptoms, including hyperventilation and bradycardia (31). Currently, there is no equivocal biomarker of an impending O<sub>2</sub>-induced seizure. HBO<sub>2</sub>-induced seizures are not deadly as long as the inspired level of

O<sub>2</sub> is lowered immediately. What is dangerous, however, are the conditions under which O<sub>2</sub> toxicity occurs such as diving at 66 fsw while breathing O<sub>2</sub>-enriched gas via a face mask or undergoing HBO<sub>2</sub> therapy for wound healing (30). HBO<sub>2</sub> is breathed in recreational diving and military diving operations using a rebreather to prevent IGN at depth, to reduce the length of decompression stops during ascent and minimize the risk of decompression sickness. HBO<sub>2</sub> therapy uses intermittent exposure to hyperoxia ranging from >1 to 3 ATA, lasting 20-30 minutes per exposure and air breaks lasting 10 minutes each (30). In either case, the potential threat of CNS oxygen toxicity limits the use of HBO<sub>2</sub>; hence, breathing protocols include significantly shortened exposures to HBO<sub>2</sub> to avoid the risk of oxygen toxicity.

***Hyperoxia and reactive species.*** The hyperexcitability of CNS oxygen toxicity is attributed to the increased production of ROS and RNS, which presumably affects multiple targets on the cell membrane due to the powerful oxidizing effects of O<sub>2</sub>. Increasing O<sub>2</sub> concentration stimulates production of ROS and RNS in experimental models *in vivo* and *in vitro* (22, 27, 48, 66), and this can have pathological or beneficial consequences depending on the O<sub>2</sub> dose. In fact, the hyperoxia-induced increase in ROS and RNS may underlie the benefits to HBO<sub>2</sub> therapy. For example, the proliferation of regenerative stem/progenitor cells in bone marrow is triggered by HBO<sub>2</sub>-induced reactive species (88, 90).

Levels of tissue PO<sub>2</sub> equivalent to HBO<sub>2</sub> (*in vivo*) can be produced at normobaric pressure in isolated cells and tissues by direct application of ≤95%O<sub>2</sub>. This produces a minimum brain slice PO<sub>2</sub> equivalent to a rat breathing >2.2 ATA O<sub>2</sub> (30, 62). Normobaric hyperoxia has rarely been used by neuroscientists as a *test* stimulus because *in vitro*

studies always use hyperoxia as the *control* O<sub>2</sub> condition (95%O<sub>2</sub>) (30); however, see (22, 27). A hyperbaric chamber is useful, and highly recommended, for *in vitro* studies of oxygen toxicity because it i) extends the testable range of PO<sub>2</sub> and ii) mimics the *in vivo* condition by providing the combined stimuli of pressure per se and increased PO<sub>2</sub>. By compressing the chamber with pure He, which mimics the effect of pressure per se (30), and varying the fractional concentration of O<sub>2</sub> and total pressure of the superfusate, the investigator can easily dissect out the independent effects of pressure per se versus PO<sub>2</sub> in the isolated CNS preparation (30, 62, 63).

An important issue to resolve for future mechanistic studies of HBO<sub>2</sub> toxicity—and mechanisms of O<sub>2</sub>-sensing in general—is what level of control O<sub>2</sub> should be employed for *in vitro* experiments since the majority of *in vitro* studies use normobaric hyperoxia (95%O<sub>2</sub>). The rationale for using 95%O<sub>2</sub> has been to avert tissue hypoxia; however, in the process, the tissue sustains “chronic” hyperoxia, which increases ROS production, stimulates neural activity, and increases cell death (22). Another potential concern is that sustained exposure to hyperoxia may be inducing oxidative preconditioning, thereby blunting subsequent neuronal responses to O<sub>2</sub> manipulation and ROS production and confounding the results of the study (4, 10, 37, 69).

***Effects of excess O<sub>2</sub> on membrane lipids and protein oxidation.*** Physical changes in the plasma membrane increase proportionally as the dose of oxygen increases, especially under hyperbaric pressure (21). Cellular membranes are a major target for ROS and RNS because of the high concentration of oxidizable membrane-bound proteins and PUFAs. Membrane PUFAs are unique fatty acids because they significantly alter basic membrane properties (visco-elasticity) and resident protein functions (77, 83). The

concentration of PUFAs in the membrane has been proposed to regulate the formation, composition and distribution of lipid microdomains (lipid rafts) on the membrane surface (76, 83), which are essentially sphingolipid and cholesterol rich platforms for cellular signal transduction, including ion channels, various transporters, G-proteins and kinases (2, 34). In addition, lipid rafts regulate cytoskeletal organization and lipid trafficking (64). Furthermore, oxidation of PUFAs containing three or more double bonds produces oxidized short chain fatty acid derivatives and the byproduct malondialdehyde (MDA). MDA increases in response to HBO<sub>2</sub> *in vivo* (7) and *in vitro* (21) and has profound effects on the physio-chemical properties of the membrane (79, 93). MDA can mediate cross-linking and polymerization of membrane lipids and membrane-embedded proteins, ultimately affecting membrane fluidity, elastic compressibility and permeability (36).

Oxidation of cellular proteins may precede membrane lipid peroxidation (25), suggesting that membrane proteins are even more susceptible to HBO<sub>2</sub> than membrane lipids. The functional consequences of membrane protein oxidation are profound, and depend on several factors including amino acid composition, efficacy of mechanisms for reversal and repair and the molecular location of the susceptible amino acids (*e.g.* intracellular or extracellular). Proteins easily oxidized have sulfur containing amino acids and/or unsaturated bonds and include tyrosine, phenylalanine, tryptophane, histidine, methionine and cysteine, all present in various ion channels, enzymes and ion transporters regulating membrane function (53). Cells with a high concentration of proteins in the membrane, like red blood cells, for example, become more fragile and susceptible to damage when exposed to hyperoxia (99). Chemical oxidants that target methionine and cysteine (chloramine-T and *N*-chlorosuccinimide) residues mimic the

stimulatory effects of HBO<sub>2</sub> on firing rate and membrane conductance in brain stem neurons (30, 31).

In summary, membrane-associated lipids and protein molecules are all regulated by oxygen availability and thus profoundly altered under conditions of HBO<sub>2</sub>. Thus HBO<sub>2</sub>-induced oxidation of membrane lipids and protein could perturb membrane-dependent functions that regulate cellular excitability (22, 30, 31, 62) and viability in neurons (22).

***HBO<sub>2</sub> effects on plasma membrane ultrastructure.*** The distinct ultrastructural feature observed at the level of the plasma membrane in response to HBO<sub>2</sub> is membrane surface blebbing; small protrusions of the plasma membrane that range in diameter from 50-200 nm, and which correlate with elevated membrane lipid peroxidation (21). Although little is known about the mechanistic nature of bleb formation, the phenomenon occurs in response to oxidative stress (101), and is triggered by elevated calcium and ROS production (49). In the context of our present discussion, it is useful therefore to consider the *possible* mechanisms by which HBO<sub>2</sub> may induce bleb formation as a means to facilitate the design of future studies addressing the molecular and cellular mechanisms of O<sub>2</sub> toxicity.

One possible scenario is that HBO<sub>2</sub>-induced membrane blebbing occurs via altered membrane phospholipid organization (55, 71) and changes in plasma membrane fluidity (11, 65, 93). In addition, HBO<sub>2</sub> could promote membrane blebbing via the oxidation of cytoskeletal proteins (57) and membrane-cytoskeleton bonds such as adhesion molecules (78). Thus, lipid-protein oxidation, especially at the tethering of

membrane-cytoskeleton bonds, could weaken the membrane causing the outward cytoplasmic pressure to form the protruding membrane surface blebs (23). In fact, it has been shown that HBO<sub>2</sub>-induced nitric oxide production inhibits neutrophil beta 2 integrin function by inhibiting membrane-associated cyclic GMP synthesis (5, 91) and causing S-nitrosylation of cytoskeletal actin (89). Similar alterations of integrin function in neurons could conceivably trigger changes in membrane morphology by weakening lipid-protein interactions and affecting cytoskeleton rearrangement. For example, transmission electron microscopy reveals that blebs are comprised of patches of membrane that have completely separated from the underlying cytoskeleton and are devoid of any cytoskeletal proteins (23). However, blebs retain integral proteins (*e.g.* ion channels), neurotransmitter receptors and glycoproteins that have important neuromodulatory function (86). Blebs also contain aggregates of cytotoxic proteins that are released during early stages of neuronal degeneration induced by hyperexcitability/excitotoxicity (49). Finally, an alternative explanation for membrane blebbing is that oxidative stress increases the formation of lipid rafts that have a tendency to attract protein aggregates, which accumulate and are visualized as blebs on the superficial cell membrane (19, 59, 103). Taken together, HBO<sub>2</sub>-induced bleb formation may be a sign of membrane destabilization from oxidative stress, possibly creating shear stress on proteins regulating neuronal excitability. However, this structure-function relationship has not been established, so future studies linking HBO<sub>2</sub>-induced bleb formation (21) to changes in neuronal excitability are needed, as well as studies to characterize the mechanism of bleb formation and its degree of reversibility once O<sub>2</sub> is lowered.

***Targeting the membrane to extend tolerance to hyperoxia.*** A significant portion of the plasma membrane is comprised of PUFAs (~50%)(81). Thus, oxidation of PUFAs could affect a variety of membrane physical properties, including fluidity, thickness, permeability, protein function and bleb formation (82). Plasma membrane fluidity and permeability decreased significantly in aged rats and in rats exposed to hyperoxia, which was due to an increased ratio of cholesterol to phospholipids and oxidation of docosahexaenoic acid (DHA) (93). All of this is consistent with the finding that HBO<sub>2</sub> increases membrane lipid peroxidation (21). These data support the notion that supplemental DHA increases neuronal membrane synthesis and membrane fluidity in animals (42, 72) and cell culture preparations (14, 16). Therefore, under conditions of oxidative stress, supplemental DHA may stabilize the membrane by replacing oxidized membrane PUFAs (82).

Antioxidant protection (exogenous and endogenous) can be an effective means to protect the plasma membrane from HBO<sub>2</sub> (21, 30, 31, 58), but it is unlikely that antioxidants alone can provide adequate protection since, under the appropriate conditions, certain agents can also act as pro-oxidants. Isomers of vitamin E (Trolox-C) are particularly effective at stabilizing membranes by scavenging lipid peroxy radicals and forming stabilizing complexes with phospholipids and free fatty acids (97). This unique property of Trolox-C may explain why it reduces membrane surface blebbing in cells exposed to HBO<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> (21). Membrane stabilization under conditions of oxidative stress is one strategy to delay the onset of CNS oxygen toxicity (seizures). Endogenous antioxidant mechanisms can be augmented through oxidative preconditioning (4, 37, 69), and this may enhance protection against oxygen toxicity (10).

A potentially effective countermeasure against oxygen toxicity could be the use of exogenous antioxidants in conjunction with various protocols of oxidative preconditioning, but this hypothesis will need to be tested.

***Effects of redox stress during hyperoxia on cell membrane nanostructure and excitability.*** The effects of hyperoxia on neuronal activity are reviewed elsewhere (30, 31). Hyperoxia acts as a general stimulant of neuronal activity, decreasing net membrane conductance and stimulating firing rate (22, 30). Both effects of HBO<sub>2</sub> are blocked by the antioxidant Trolox-C (30), which is reported to also decrease membrane lipid peroxidation and membrane blebbing during graded hyperoxia (21). Based on the above discussion, it seems appropriate to consider the effects of redox stress on membrane nanostructure when designing future experiments to test new models for cellular oxygen toxicity. For example, decreased membrane conductance during hyperoxia describes net closure of ion channels, presumably decreasing outward (K<sup>+</sup>) and/or inward (Cl<sup>-</sup>) currents causing depolarization and increased firing rate (30, 31). Ion channel gating could be affected *directly* by redox mechanisms, or *indirectly* by membrane perturbations involving: i) oxidation of membrane-cytoskeletal bonds; ii) distortion via membrane lipid peroxidation and blebbing (21); and/or iii) membrane expansion as molecular O<sub>2</sub> diffuses into the bilayer as dictated by its relatively high lipid solubility (8). In future studies, it will be important to identify the subtle, graded effects of ROS/RNS on membrane structure and function over a continuum of PO<sub>2</sub> that spans normoxia through hyperoxia to identify the potentially therapeutic, neuroprotective effects of redox stimuli (4, 10, 33, 37) versus their deleterious effects in oxygen toxicity (3).

## INERT GAS NARCOSIS

Inert gases are non-reactive gases at normobaric pressure; however, with increasing  $P_B$ , “inert” gases become narcotic (8, 30, 102). Nitrogen is a significant inert gas in diving medicine because it comprises ~79% of air. The other important inert gas is He, which is substituted for  $N_2$  as the balance gas in the breathing mixture at >6 ATA (Heliox) (30). Additional inert gases are the other Noble gases, including argon, neon, krypton and Xenon.

***Under what conditions does IGN occur?*** The effects of  $HBN_2$  on mental awareness and neuronal activity have been reviewed elsewhere (30). Nitrogen narcosis occurs when breathing air at ~4 ATA (99 fsw) and has been called “Rapture of the Deep”. The symptoms include escalating euphoria, reduced mental processing, and impaired neuromuscular coordination, very much resembling alcohol intoxication or the early stages of anesthesia. Symptoms worsen with increasing depth until consciousness is lost at  $P_B >10$  ATA (30). Onset of narcosis is relatively rapid, but body composition and prior strenuous tasks can increase the onset and severity of symptoms. Conditions that increase cerebral blood flow, such as  $CO_2$  retention, will enhance uptake of inert gas within the CNS and hasten onset and severity of the symptoms (30).

***Effects of inert gases on membrane lipids and proteins.*** The role of chemically inert gases in the induction of gas narcosis is well defined for the intact organism (12, 70). In short, narcotic potency is directly related to the gas’ molecular weight (MW) and lipid solubility as follows:

He (2) < Ne (10) < N<sub>2</sub> (14) < Ar (18) < Kr (36) < Xe (54)

→ → → increasing lipid solubility and narcotic potency → → →

For example, air (0.79 N<sub>2</sub>) elicits neurocognitive deficits at ~4 ATA, whereas Xe modifies cellular activity (0.85 ATA Xe) and whole animal neurocognition (0.35ATA Xe) at normobaric pressure (6, 9, 32). Conversely, He, which has the lowest lipid solubility of all inert gases, has no known narcotic actions on the CNS in the range of physiological tolerable pressures (30). This is why pure He is used to compress the hyperbaric chamber to study cellular barosensitivity (28, 30, 62, 63).

The molecular and cellular mechanisms responsible for IGN are largely unknown, and currently there is no unifying theory of narcosis. Because the symptoms of narcosis resemble anesthesia, the two conditions are often considered together regarding their mechanisms. The fact that narcosis and anesthesia exhibit pressure reversal (see below), further supports this conclusion (102). Two general theories of narcosis/anesthesia have been proposed: lipids versus proteins. In general, the lipid theory of narcosis states that gases with the greatest lipid solubility will have the greatest effect on the bilayer, increasing membrane volume and fluidity (56, 102). For example, laser scattering microscopy revealed an increase in membrane fluidity during exposure to normobaric Xe (greatest lipid solubility) and ethanol (40). The lipid theory of narcosis predicts that an increase in membrane volume by inert gas will induce conformational changes in various membrane-bound proteins and induce narcosis (8, 102). Alternatively, the protein theory of narcosis/anesthesia proposes that ion channels and post-synaptic receptors are the primary site of narcotic/anesthetic actions (35, 102). It has been proposed that during

IGN, N<sub>2</sub> gas molecules bind to specific hydrophobic sites inside the post-synaptic ion channels of glutamate receptors (92).

***Effects of inert gases on neuronal excitability.*** Regardless of the exact target affected by HBN<sub>2</sub>, electrophysiological studies indicate that inert gases affect electrical signaling at hyperbaric pressure. Hyperbaric air increased the rate of depolarization and repolarization of the action potential, suggesting that HBN<sub>2</sub> increases Na<sup>+</sup> and K<sup>+</sup> channels (15, 73). HBN<sub>2</sub> also inhibited synaptic transmission (15, 73). Exposure to normobaric Xe produced significant inhibition of voltage-gated Ca<sup>2+</sup> and K<sup>+</sup> channels (44, 100) and ligand-gated ionic currents (32, 67). Future electrophysiological studies will need to determine how inert gases, over a range of pressures, affect neuronal excitability. Likewise, hyperbaric AFM (20) will be useful for studying changes in plasma membrane fluidity and protein conformations in real time as a function of inert gas pressure to test the foregoing two sets of hypotheses.

***Pressure reversal of narcosis/anesthesia.*** Hyperbaric pressure (see below) antagonizes the narcotic actions of inert gases on the CNS and vice versa (12, 56, 70). For this reason alone, N<sub>2</sub> is used in trimix breathing gas (O<sub>2</sub>-He/H<sub>2</sub>-N<sub>2</sub>) for deep ranging technical dives (≥15 ATA) to ameliorate HPNS (30). At the cellular level, hyperbaric pressure reverses the narcotic actions of inert gas on synaptic transmission (73), action potential frequency (15), and ionic currents (24). The specific membrane mechanism responsible for this phenomenon, however, is unknown. Włodarczyk et al. (102) recently summarized current theories of how pressure reversal of narcosis/anesthesia may occur. The controversy, as expected, revolves around the nanostructure involved—lipids versus proteins—and how pressure reverses narcosis. The lipid theory proposes that pressure

and narcotic gases have antagonistic effects on membrane volume, effectively canceling out the effects of the other. The protein theory proposes that narcotic gas molecules, which bind to membrane proteins and induce narcosis (92), are “dislodged” by pressure thereby reversing narcosis. Clearly, this is an area requiring additional work, but one which will provide insight into the general mechanisms of anesthesia.

### **MODERATE LEVELS OF HYPERBARIC PRESSURE (<10 ATA)**

None of the foregoing problems would occur unless O<sub>2</sub> and N<sub>2</sub> are breathed at hyperbaric pressure. One must, therefore, consider the additional stimulus of hydrostatic pressure. At the systems level, a pressure-applied force against the surface of the body rapidly equilibrates throughout the tissues. At the cellular level, however, it has been proposed that the various non-fluid, nanostructures comprising the cell membrane undergo differential compression. This is hypothesized to produce localized shear and strain forces between adjoining nanostructures (lipid bilayer, cytoskeleton and membrane-bound proteins) that perturb ion channel gating (30, 50, 52). In this context, it is worth mentioning that mechanosensitive ion channels have also been identified, which respond to expansion and contraction of the cell membrane (60). Thus, changes in bilayer volume may activate mechanosensitive ion channels via shear forces generated by membrane-cytoskeletal interactions. Neuronal barosensitivity (<10 ATA), therefore, may be a general form of mechanosensitivity. What is interesting is that barosensitivity does not correlate with sensitivity to either hyperoxia or hypercapnia in brain stem neurons (62, 63). Clearly, pressure per se is a modulating stimulus different from that of gas

partial pressure, which affects a specific population of neurons, at least in the brain stem (30).

An interesting model for studying the membrane effects of hydrostatic pressure is the European eel (*Anguilla anguilla* L.), which employs physiological adaptations (metamorphosis) prior to deep sea migrations to depths in excess of 100-200 ATA. Enhancement of mitochondrial membrane fluidity appears to be the underlying adaptation essential for tolerance to high hydrostatic pressure (96). These *pre-adaptations to elevated hydrostatic pressure* include increased membrane PUFA content and decreased membrane cholesterol, which offsets the pressure-induced increase in phospholipid order that imparts a membrane rigidifying effect (87). Increased membrane fluidity improves oxidative phosphorylation and reduces electron leak (74), perhaps by enhancing the function of enzymatic complexes embedded in the inner mitochondrial membrane (96).

## **PERSPECTIVE**

The neurological consequences of breathing hyperbaric gases are directly related to the gas' partial pressure, lipid solubility, and capacity to alter the physicochemical properties of adjoining membrane nanostructures through oxidation and mechanical perturbation. All of these effects are enhanced by increasing total gas pressure. The ensuing neurological consequences can be clinically useful by promoting wound healing (30, 88), oxidative preconditioning (4, 10, 37) and induction of anesthesia (102). Alternatively, the effects are pathologically damaging causing CNS oxygen toxicity (seizure, oxidative stress and cell death) and CO<sub>2</sub> toxicity/narcosis (3, 30). The diversity

of stimuli and membrane mechanisms that are activated by hyperbaric gases, therefore, have biomedical relevance for diving and hyperbaric medicine plus any non-diving discipline interested in membrane models of redox signaling, oxidative stress (33) and general anesthesia (102). Continued development and use of hyperbaric research tools that enable probing of membrane nanostructure and physicochemical properties in real time (e.g. (20)), while manipulating gas partial pressure and  $P_B$ , are anticipated to provide rich insight into the potential clinical application of hyperbaric gases and how they modulate neurological function.

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