J Appl Physiol 106: 996–1003, 2009.
First published September 27, 2008; doi:10.1152/japplphysiol.91070.2008.

HIGHLIGHTED TOPIC | *The Physiology and Pathophysiology of the Hyperbaric and Diving Environments*

Effects of hyperbaric gases on membrane nanostructure and function in neurons

Dominic P. D'Agostino,[1] Denis G. Colomb Jr.,[2] and Jay B. Dean[1]

[1]*Hyperbaric Biomedical Research Laboratory, Department of Molecular Pharmacology and Physiology, College of Medicine, University of South Florida, Tampa; and* [2] *Hyperbaric Electrophysiology Laboratory, Biomedical Department, Navy Experimental Diving Unit, Panama City, Florida*

Submitted 8 August 2008; accepted in final form 18 September 2008

D'Agostino DP, Colomb DG Jr, Dean JB. Effects of hyperbaric gases on membrane nanostructure and function in neurons. *J Appl Physiol* 106: 996–1003, 2009. First published September 27, 2008; doi:10.1152/japplphysiol.91070.2008.—This mini-review summarizes current ideas of how hyperbaric gases (>1–10 atmospheres absolute) 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 make each respective nanostructure a potential target for modulation by hyperbaric gases. Depending on the composition of the gas mixture, the relative concentrations of O_2 and inert gas, and total barometric pressure, the net effect of a particular gas on the cell membrane will be determined by the gas' *1*) lipid solubility, *2*) ability to oxidize lipids and proteins (O_2), and *3*) 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.

anesthesia; barosensitivity; free radicals; inert gas narcosis; nitrogen narcosis; oxidative stress; oxygen toxicity

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)[1]] down to a maximum pressure of ~70 ATA, which is equivalent to ~2,300 feet of seawater (fsw) (26, 30).[2] 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_2 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 O_2 (HBO_2) and hyperbaric N_2 (HBN_2). This means decreasing the fractional concentration of N_2 in air to <0.79 to avert the euphoric irrationality of inert gas narcosis (IGN), otherwise known as N_2 narcosis, and carefully regulating inspired O_2 to avoid the violent, uncontrollable seizures of central nervous system (CNS) O_2 toxicity (reviewed in Ref. 30). At even greater depths, in the absence of CNS O_2 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 responsible for each neurological condition is readily apparent when the suspected underlying causes are considered: CNS O_2 toxicity is attributed to the harmful effects of various species of reactive O_2 species (ROS) and reactive N_2 species (RNS), which together are called reactive species (3, 30); IGN is attributed to the narcotic action of high-pressure N_2 (30, 102); and HPNS is attributed to the direct effects of hydrostatic compression of the CNS in the absence of narcosis (30, 84). Although 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).

[1] atmosphere absolute (ATA) is barometric pressure (P_B) at sea level. 1 ATA = 14.7 psi = 760 mmHg = 0.1 MPa.

[2] 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.

Address for reprint requests and other correspondence: J. B. Dean, Dept. of Molecular Pharmacology and Physiology, College of Medicine, MDC 8, 12901 Bruce B. Downs Blvd., Tampa, FL 33612 (e-mail: jdean@health.usf.edu).

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 O_2 toxicity and IGN and review the effects of HBO_2, 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 O_2 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., Refs. 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).[3] Previous studies have tended to focus on the effects of extreme hyperbaria, usually ≥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 ~3–4 ATA and do 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 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' *1*) lipid solubility (8, 102), *2*) ability to oxidize lipids and proteins (O_2) (3, 33, 88), and *3*) capacity through compression to generate localized shear and strain forces between membrane-bound proteins and lipids with differential compressibility (50, 52).

[3] 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 hydreliox gas mixtures (30).

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 inward and the hydrophilic phosphate groups facing outward toward 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 ~5–7 nm (50–70 Å), and it is 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 Ca^{2+} and associated downstream signaling pathways, and they 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 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 make each respective nanostructure a potential target of molecular O_2, molecular N_2, reactive species and physical pressure under hyperbaric conditions as dictated by the composition of breathing gas.

HBO_2 TOXICITY

O_2 toxicity occurs under what conditions? CNS O_2 toxicity occurs when breathing pure O_2 at greater than 2–3 ATA. O_2 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_2-induced seizure. HBO_2-induced seizures are not deadly as long as the inspired level of O_2 is lowered immediately. What is dangerous, however, are the conditions under which O_2 toxicity occurs such as diving at 66 fsw while breathing O_2-enriched gas via a face mask or undergoing HBO_2 therapy for wound healing (30). HBO_2 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_2 therapy uses intermittent exposure to hyperoxia ranging from >1 to 3 ATA, lasting 20–30 min per exposure, and air breaks lasting 10 min each (30). In either case, the potential threat of CNS O_2 toxicity limits the use of HBO_2; hence, breathing protocols include significantly shortened exposures to HBO_2 to avoid the risk of CNS O_2 toxicity.

Hyperoxia and reactive species. The hyperexcitability of CNS O_2 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_2. Increasing O_2 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_2 dose. In fact, the hyperoxia-induced increase in ROS and RNS may underlie the benefits to HBO_2 therapy. For example, the proliferation of regenerative stem/progenitor cells in bone marrow is triggered by HBO_2-induced reactive species (88, 90).

Levels of tissue P_{O_2} equivalent to HBO_2 (in vivo) can be produced at normobaric pressure in isolated cells and tissues by direct application of $\leq$95% O_2. This produces a minimum brain slice P_{O_2} equivalent to a rat breathing >2.2 ATA O_2 (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_2 condition (95% O_2) (30) (however, see Refs. 22, 27). A hyperbaric chamber is useful, and highly recommended, for in vitro studies of O_2 toxicity because it *1*) extends the testable range of P_{O_2} and *2*) mimics the in vivo condition by providing the combined stimuli of pressure per se and increased P_{O_2}. By compressing the chamber with pure He, which mimics the effect of pressure per se (30), and varying the fractional concentration of O_2 and total pressure of the superfusate, the investigator can easily dissect out the independent effects of pressure per se vs. P_{O_2} in the isolated CNS preparation (30, 62, 63).

An important issue to resolve for future mechanistic studies of HBO_2 toxicity (and mechanisms of O_2 sensing in general) is what level of control O_2 should be employed for in vitro experiments because the majority of in vitro studies use normobaric hyperoxia (95% O_2). The rationale for using 95% O_2 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_2 manipulation and ROS production and confounding the results of the study (4, 10, 37, 69).

Effects of excess O_2 on membrane lipids and protein oxidation. Physical changes in the plasma membrane increase proportionally as the dose of O_2 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 (viscoelasticity) 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 by-product malondialdehyde (MDA). MDA increases in response to HBO_2 in vivo (7) and in vitro (21) and has profound effects on the physiochemical 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_2 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_2 on firing rate and membrane conductance in brain stem neurons (30, 31).

In summary, membrane-associated lipids and protein molecules are all regulated by O_2 availability and thus profoundly altered under conditions of HBO_2. Thus HBO_2-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_2 effects on plasma membrane ultrastructure. The distinct ultrastructural feature observed at the level of the plasma membrane in response to HBO_2 is membrane surface blebbing; small protrusions of the plasma membrane that range in diameter from 50 to 200 nm and that 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 it 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_2 may induce bleb formation as a means to facilitate the design of future studies addressing the molecular and cellular mechanisms of O_2 toxicity.

One possible scenario is that HBO_2-induced membrane blebbing occurs via altered membrane phospholipid organization (55, 71) and changes in plasma membrane fluidity (11, 65, 93). In addition, HBO_2 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 show that HBO_2-induced nitric oxide production inhibits neutrophil β_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 composed 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_2-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_2-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_2 is lowered.

Targeting the membrane to extend tolerance to hyperoxia. A significant portion of the plasma membrane is composed 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_2 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_2 (21, 30, 31, 58), but it is unlikely that antioxidants alone can provide adequate protection because, under the appropriate conditions, certain agents can also act as prooxidants. Isomers of vitamin E (Trolox C) are particularly effective at stabilizing membranes by scavenging lipid peroxyl 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_2 and H_2O_2 (21). Membrane stabilization under conditions of oxidative stress is one strategy to delay the onset of CNS O_2 toxicity (seizures). Endogenous antioxidant mechanisms can be augmented through oxidative preconditioning (4, 37, 69), and this may enhance protection against O_2 toxicity (10). A potentially effective countermeasure against O_2 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_2 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 O_2 toxicity. For example, decreased membrane conductance during hyperoxia describes net closure of ion channels, presumably decreasing outward (K^+) and/or inward (Cl^-) 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 *1*) oxidation of membrane-cytoskeletal bonds; *2*) distortion via membrane lipid peroxidation and blebbing (21); and/or *3*) membrane expansion as molecular O_2 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 P_{O_2} that spans normoxia through hyperoxia to identify the potentially therapeutic, neuroprotective effects of

redox stimuli (4, 10, 33, 37) vs. their deleterious effects in O_2 toxicity (3).

INERT GAS NARCOSIS

Inert gases are nonreactive gases at normobaric pressure; however, with increasing P_B, "inert" gases become narcotic (8, 30, 102). N_2 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 Ar, Ne, Kr, and Xe.

Under what conditions does IGN occur? The effects of HBN_2 on mental awareness and neuronal activity have been reviewed elsewhere (30). N_2 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 and lipid solubility as follows (listed in order of increasing lipid solubility and narcotic potency):

$$\text{He}\,(2) < \text{Ne}\,(10) < \text{N}_2\,(14) < \text{Ar}\,(18) < \text{Kr}\,(36) < \text{Xe}\,(54)$$

For example, air (0.79 N_2) elicits neurocognitive deficits at ~4 ATA, whereas Xe modifies cellular activity (0.85 ATA Xe) and whole animal neurocognition (0.35 ATA 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 vs. 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 postsynaptic receptors are the primary site of narcotic/anesthetic actions (35, 102). It has been proposed that during IGN, N_2 gas molecules bind to specific hydrophobic sites inside the postsynaptic ion channels of glutamate receptors (92).

Effects of inert gases on neuronal excitability. Regardless of the exact target affected by HBN_2, 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_2 increases Na^+ and K^+ channels (15, 73). HBN_2 also inhibited synaptic transmission (15, 73). Exposure to normobaric Xe produced significant inhibition of voltage-gated Ca^{2+} and K^+ 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. Similarly, 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_2 is used in trimix breathing gas (O_2-He/H_2-N_2) 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. Wlodarczyk 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 vs. 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 it is one that 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_2 and N_2 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 nonfluid, 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) before 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 preadaptations to elevated hydrostatic pressure include increased membrane PUFA content and decreased membrane cholesterol, which offset 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 O_2 toxicity (seizure, oxidative stress and cell death) and CO_2 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 nondiving 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., Ref. 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.

ACKNOWLEDGMENTS

D. P. D'Agostino and J. B. Dean gratefully acknowledge the assistance in AFM theory and application that was provided graciously by Dr. Helen McNally (Purdue Univ.), and the outstanding technical support of Carol Landon.

GRANTS

Research and ideas discussed herein are based in part on work performed in the authors' laboratories funded by the Office of Naval Research, Undersea Medicine Program: ONR Grants N000140610105 (to D. P. D'Agostino), N0001408WX20220 (to D. G. Colomb), and N000140710890 (to J. B. Dean).

DISCLOSURES

The views expressed in this article are those of the author (Dr. G. Colomb) and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government.

REFERENCES

1. **Acker T, Acker H.** Cellular oxygen sensing need in CNS function: physiological and pathological implications. *J Exp Biol* 207: 3171–3188, 2004.
2. **Ahmed SN, Brown DA, London E.** On the origin of sphingolipid/cholesterol-rich detergent-insoluble cell membranes: physiological concentrations of cholesterol and sphingolipid induce formation of a detergent-insoluble, liquid-ordered lipid phase in model membranes. *Biochemistry* 36: 10944–10953, 1997.
3. **Allen BW, Demchenko IT, Piantadosi CA.** Two faces of nitric oxide: implications for cellular mechanisms of oxygen toxicity. *J Appl Physiol* (October 9, 2008). doi:10.1152/japplphysiol.91109.2008.
4. **Arkhipenko YV, Sazontova TG, Zhukova AG.** Adaptation to periodic hypoxia and hyperoxia improves resistance of membrane structures in heart, liver, and brain. *Bull Exp Biol Med* 140: 278–281, 2005.
5. **Banick PD, Chen Q, Xu YA, Thom SR.** Nitric oxide inhibits neutrophil beta 2 integrin function by inhibiting membrane-associated cyclic GMP synthesis. *J Cell Physiol* 172: 12–24, 1997.
6. **Bedi A, McCarroll C, Murray JM, Stevenson MA, Fee JP.** The effects of subanaesthetic concentrations of xenon in volunteers. *Anaesthesia* 57: 233–241, 2002.
7. **Benedetti S, Lamorgese A, Piersantelli M, Pagliarani S, Benvenuti F, Canestrari F.** Oxidative stress and antioxidant status in patients undergoing prolonged exposure to hyperbaric oxygen. *Clin Biochem* 37: 312–317, 2004.
8. **Bennett PB, Papahadjopoulos D, Bangham AD.** The effect of raised pressure of inert gases on phospholipid membranes. *Life Sci* 6: 2527–2533, 1967.
9. **Benrath J, Kempf C, Georgieff M, Sandkuhler J.** Xenon blocks the induction of synaptic long-term potentiation in pain pathways in the rat spinal cord in vivo. *Anesth Analg* 104: 106–111, 2007.
10. **Bleiberg B, Kerem D.** Central nervous system oxygen toxicity in the resting rats: postponement by intermittent oxygen exposure. *Undersea Biomed Res* 15: 337–352, 1988.
11. **Block ER, Patel JM, Angelides KJ, Sheridan NP, Garg LC.** Hyperoxia reduces plasma membrane fluidity: a mechanism for endothelial cell dysfunction. *J Appl Physiol* 60: 826–835, 1986.
12. **Brauer RW, Hogan PM, Hugon M, Macdonald AG, Miller KW.** Patterns of interaction of effects of light metabolically inert gases with those of hydrostatic pressure as such-a review. *Undersea Biomed Res* 9: 353–396, 1982.
13. **Bretscher MS.** The molecules of the cell membrane. *Sci Am* 253: 100–108, 1985.
14. **Brown ER, Subbaiah PV.** Differential effects of eicosapentaenoic acid and docosahexaenoic acid on human skin fibroblasts. *Lipids* 29: 825–829, 1994.
15. **Bryant HJ, Blankenship JE.** Action potentials in single axons: effects of hyperbaric air and hydrostatic pressure. *J Appl Physiol* 47: 561–567, 1979.
16. **Calder PC, Yaqoob P, Harvey DJ, Watts A, Newsholme EA.** Incorporation of fatty acids by concanavalin A-stimulated lymphocytes and the effect on fatty acid composition and membrane fluidity. *Biochem J 300*: 509–518, 1994.
17. **Cooper GJ, Zhou Y, Bouyer P, Grichtchenko II, Boron WF.** Transport of volatile solutes through AQP1. *J Physiol* 542: 17–29, 2002.
18. **Cunningham CC.** Actin polymerization and intracellular solvent flow in cell surface blebbing. *J Cell Biol* 129: 1589–1599, 1995.
19. **Cuschieri J, Maier RV.** Oxidative stress, lipid rafts, and macrophage reprogramming. *Antioxid Redox Signal* 9: 1485–1498, 2007.
20. **D'Agostino DP, Dean JB.** Development of hyperbaric atomic force microscopy (HAFM) to study the effects of oxidative stress on the plasma membrane of CNS cells. *Soc Neurosci Abstr*. Program No. 395.8.
21. **D'Agostino DP, Olson JE, Dean JB.** Atomic force microscopy (AFM) analysis of lipid peroxidation following hyperoxia and hydrogen peroxide treatment in human U87 glioblastoma cells. *FASEB J* 22: 747.742, 2008.

22. **D'Agostino DP, Putnam RW, Dean JB.** Superoxide ($O_2^{\bullet -}$) production in CA1 neurons of rat hippocampal slices exposed to graded levels of oxygen. *J Neurophysiol* 98: 1030–1041, 2007.
23. **Dai J, Sheetz MP.** Membrane tether formation from blebbing cells. *Biophys J* 77: 3363–3370, 1999.
24. **Davies DL, Alkana RL.** Benzodiazepine agonist and inverse agonist coupling in $GABA_A$ receptors antagonized by increased atmospheric pressure. *Eur J Pharmacol* 469: 37–45, 2003.
25. **Davies KJ, Goldberg AL.** Oxygen radicals stimulate intracellular proteolysis and lipid peroxidation by independent mechanisms in erythrocytes. *J Biol Chem* 262: 8220–8226, 1987.
26. **Dean JB, D'Agostino DP.** Pressure effects on human physiology. In: *Physiology and Medicine of Hyperbaric Oxygen Therapy,* edited by Neuman TS and Thom SR. Philadelphia, PA: Elsevier, 2008, chapt. 10, p. 189–202.
27. **Dean JB, D'Agostino DP, Landon CL, Matott MP, Hartzler LK, Putnam RW.** Superoxide production increases in nucleus tractus solitarius (NTS) neurons in rat brain slices during acute normobaric hyperoxia and hypoxia. *Soc Neurosci Abstr.* Program No. 481.20.
28. **Dean JB, Mulkey DK.** Continuous intracellular recording from mammalian neurons exposed to hyperbaric helium, oxygen, or air. *J Appl Physiol* 89: 807–822, 2000.
29. **Dean JB, Mulkey DK, Arehart JT.** Details on building a hyperbaric chamber for intracellular recording in brain tissue slices. *J Appl Physiol* 89: 807–822, 2000. [Supplemental material (http://jap.physiology.org/cgi/content/full/889/802/807/DC801)].
30. **Dean JB, Mulkey DK, Garcia AJ III, Putnam RW, Henderson RA III.** Neuronal sensitivity to hyperoxia, hypercapnia, and inert gases at hyperbaric pressures. *J Appl Physiol* 95: 883–909, 2003.
31. **Dean JB, Mulkey DK, Henderson IIIRA, Potter SJ, Putnam RW.** Hyperoxia, reactive O_2 species, and hyperventilation: O_2 sensitivity of brain stem neurons. *J Appl Physiol* 96: 784–791, 2004.
32. **Dinse A, Fohr KJ, Georgieff M, Beyer C, Bulling A, Weigt HU.** Xenon reduces glutamate-, AMPA-, and kainate-induced membrane currents in cortical neurones. *Br J Anaesth* 94: 479–485, 2005.
33. **Droge W.** Free radicals in the physiological control of cell function. *Physiol Rev* 82: 47–95, 2001.
34. **Edidin M.** The state of lipid rafts: from model membranes to cells. *Annu Rev Biophys Biomol Struct* 32: 257–283, 2003.
35. **Franks NP, Lieb WR.** Molecular mechanisms of general anesthesia. *Nature* 300: 487–493, 1982.
36. **Freeman BA, Crapo JD.** Biology of disease: free radicals and tissue injury. *Lab Invest* 47: 412–426, 1982.
37. **Freiberger JJ, Suliman HB, Sheng H, McAdoo J, Piantadosi CA, Warner DS.** A comparison of hyperbaric oxygen versus hypoxic cerebral preconditioning in neonatal rats. *Brain Res* 1075: 213–222, 2006.
38. **Garcia AJ III.** *Oxygen Sensitivity and Plasticity of CA1 Neurons in the Rat Hippocampus at Normobaric and Hyperbaric Pressures* (Doctoral dissertation). Dayton, OH: Wright State University, 2006.
39. **Grenningloh G, Soehrman S, Bondallaz P, Ruchti E, Cadas H.** Role of the microtubule destabilizing proteins SCG10 and stathmin in neuronal growth. *J Neurobiol* 58: 60–69, 2004.
40. **Haidekker MA, Stevens HY, Frangos JA.** Cell membrane fluidity changes and membrane undulations observed using a laser scattering technique. *Ann Biomed Eng* 32: 531–536, 2004.
41. **Halliwell B, Gutteridge JMC.** Cellular responses to oxidative stress: adaptation, damage, repair, senescence and death. In: *Free Radicals in Biology and Medicine*. New York: Oxford University Press, 2007, p. 187–267.
42. **Hashimoto M, Hossain S, Shimada T, Shido O.** Docosahexaenoic acid-induced protective effect against impaired learning in amyloid beta-infused rats is associated with increased synaptosomal membrane fluidity. *Clin Exp Pharmacol Physiol* 33: 934–939, 2006.
43. **Horrocks LA, Farooqui AA.** Docosahexaenoic acid in the diet: its importance in maintenance and restoration of neural membrane function. *Prostaglandins Leukot Essent Fatty Acids* 70: 361–372, 2004.
44. **Huneke R, Jungling E, Skasa M, Rossaint R, Luckhoff A.** Effects of the anesthetic gases xenon, halothane, and isoflurane on calcium and potassium currents in human atrial cardiomyocytes. *Anesthesiology* 95: 999–1006, 2001.
45. **Hwang SH.** *Effects of Normobaric and Hyperbaric Oxygen on Nitric Oxide Production in Rat Brain Slices* (Masters thesis). Dayton, OH: Wright State University, 2004.
46. **Jacobson K, Sheets ED, Simson R.** Revisiting the fluid mosaic model of membranes. *Science* 268: 1441–1442, 1995.
47. **Jekely G.** Origin of eukaryotic endomembranes: a critical evaluation of different model scenarios. *Adv Exp Med Biol* 607: 38–51, 2007.
48. **Kaindl AM, Sifringer M, Zabel C, Nebrich G, Wacker MA, Felderhoff-Mueser U, Endesfelder S, von der Hagen M, Stefovska V, Klose J, and Ikonomidou C.** Acute and long-term proteome changes induced by oxidative stress in the developing brain. *Cell Death Differ* 13: 1097–1109, 2006.
49. **Lesort M, Terro F, Esclaire F, Hugon J.** Neuronal APP accumulates in toxic membrane blebbings. *J Neural Transm* 104: 497–513, 1997.
50. **Macdonald AG.** Hydrostatic pressure as an environmental factor in life processes. *Comp Biochem Physiol* 116A: 291–297, 1997.
51. **Macdonald AG.** Ion channels under high pressure. *Comp Biochem Physiol A Mol Integr Physiol* 131: 587–593, 2002.
52. **Macdonald AG, Fraser PJ.** The transduction of very small hydrostatic pressures. *Comp Biochem Physiol A Mol Integr Physiol* 122: 13–36, 1999.
53. **Maher P.** Redox control of neural function: background, mechanisms, and significance. *Antioxid Redox Signal* 8: 1941–1970, 2006.
54. **McIntosh TJ, Simon SA.** Roles of bilayer material properties in function and distribution of membrane proteins. *Annu Rev Biophys Biomolecular Structure* 35: 177–198, 2006.
55. **Megli FM, Russo L.** Different oxidized phospholipid molecules unequally affect bilayer packing. *Biochim Biophys Acta*, 1778: 143–152, 2007.
56. **Miller KW.** Inert gas narcosis, the high pressure neurological syndrome, and the critical volume hypothesis. *Science* 185: 867–869, 1974.
57. **Miyoshi H, Umeshita K, Sakon M, Imajoh-Ohmi S, Fujitani K, Gotoh M, Oiki E, Kambayashi J, Monden M.** Calpain activation in plasma membrane bleb formation during tert-butyl hydroperoxide-induced rat hepatocyte injury. *Gastroenterology* 110: 1897–1904, 1996.
58. **Mollaoglu H, Topal T, Ozler M, Uysal B, Reiter RJ, Korkmaz A, Oter S.** Antioxidant effects of melatonin in rats during chronic exposure to hyperbaric oxygen. *J Pineal Res* 42: 50–54, 2007.
59. **Morgan MJ, Kim YS, Liu Z.** Lipid rafts and oxidative stress-induced cell death. *Antioxid Redox Signal* 9: 1471–1484, 2007.
60. **Morris CE.** Mechanoprotection of the plasma membrane in neurons and other non-erythroid cells by the spectrin-based membrane skeleton. *Cell Mol Biol Lett* 6: 703–720, 2001.
61. **Mulkey DK, Henderson RA III, Olson JE, Putnam RW, Dean JB.** Oxygen measurement in brain stem slices exposed to normobaric hyperoxia and hyperbaric oxygen. *J Appl Physiol* 90: 1887–1899, 2001.
62. **Mulkey DK, Henderson RA III, Putnam RW, Dean JB.** Hyperbaric oxygen and chemical oxidants stimulate CO_2/H^+-sensitive neurons in rat brain stem slices. *J Appl Physiol* 95: 910–921, 2003.
63. **Mulkey DK, Henderson RA III, Putnam RW, Dean JB.** Pressure ($\leq$4 ATA) increases membrane conductance and firing rate in the rat solitary complex. *J Appl Physiol* 95: 922–930, 2003.
64. **Munro S.** Lipid rafts: elusive or illusive? *Cell* 115: 377–388, 2003.
65. **Patel JM, Block ER.** The effect of oxidant gases on membrane fluidity and function in pulmonary endothelial cells. *Free Radic Biol Med* 4: 121–134, 1988.
66. **Pichiule P, Chavez JC, LaManna JC.** Oxygen and oxidative stress modulate the expression of uncoupling protein-5 in vitro and in vivo. *Adv Exp Med Biol* 540: 103–107, 2003.
67. **Plested AJ, Wildman SS, Lieb WR, Franks NP.** Determinants of the sensitivity of AMPA receptors to xenon. *Anesthesiology* 100: 347–358, 2004.
68. **Prabhakar NR, Kumar GK.** Oxidative stress in the systemic and cellular responses to intermittent hypoxia. *Biol Chem* 385: 217–221, 2004.
69. **Ravati A, Ahlemeyer B, Becker A, Krieglstein J.** Preconditioning-induced neuroprotection is mediated by reactive oxygen species. *Brain Research* 866: 23–32, 2000.
70. **Rostain JC, Balon N.** Recent neurochemical basis of inert gas narcosis and pressure effects. *Undersea Hyperb Med* 33: 197–204, 2006.
71. **Sabatini K, Mattila JP, Megli FM, Kinnunen PK.** Characterization of two oxidatively modified phospholipids in mixed monolayers with DPPC. *Biophys J* 90: 4488–4499, 2006.
72. **Sakamoto T, Cansev M, Wurtman RJ.** Oral supplementation with docosahexaenoic acid and uridine-5′-monophosphate increases dendritic spine density in adult gerbil hippocampus. *Brain Res* 1182: 50–59, 2007.

73. **Sauter JF.** Electrophysiological activity of a mammalian sympathetic ganglion under hydrostatic and inert gas pressure. *Neuropharmacology* 18: 77–81, 1979.
74. **Scaion D, Vettier A, Sebert P.** Pressure and temperature interactions on aerobic metabolism in migrating silver eels: results in vitro. *Undersea Hyperb Med* 35: 27–33, 2008.
75. **Shahin V, Barrera NP.** Providing unique insight into cell biology via atomic force microscopy. *Int Rev Cytol* 265: 227–252, 2008.
76. **Shaikh SR, Brzustowicz MR, Gustafson N, Stillwell W, Wassall SR.** Monounsaturated PE does not phase-separate from the lipid raft molecules sphingomyelin and cholesterol: role for polyunsaturation? *Biochemistry* 41: 10593–10602, 2002.
77. **Shaikh SR, Cherezov V, Caffrey M, Stillwell W, Wassall SR.** Interaction of cholesterol with a docosahexaenoic acid-containing phosphatidylethanolamine: trigger for microdomain/raft formation? *Biochemistry* 42: 12028–12037, 2003.
78. **Sheetz MP, Sable JE, Dobereiner HG.** Continuous membrane-cytoskeleton adhesion requires continuous accommodation to lipid and cytoskeleton dynamics. *Annu Rev Biophys Biomol Struct* 35: 417–434, 2006.
79. **Sheridan NP, Block ER.** Plasma membrane fluidity measurements in intact endothelial cells: effect of hyperoxia on fluorescence anisotropies of 1-[4-(trimethylamino)phenyl]-6-phenyl hexa-1,3,5-triene. *J Cell Physiol* 134: 117–123, 1988.
80. **Singer SJ, Nicolson GL.** The fluid mosaic model of the structure of cell membranes. *Science* 175: 720–731, 1972.
81. **Singh M.** Essential fatty acids, DHA and human brain. *Indian J Pediatr* 72: 239–242, 2005.
82. **Stillwell W, Shaikh SR, Zerouga M, Siddiqui R, Wassall SR.** Docosahexaenoic acid affects cell signaling by altering lipid rafts. *Reprod Nutr Dev* 45: 559–579, 2005.
83. **Stillwell W, Wassall SR.** Docosahexaenoic acid: membrane properties of a unique fatty acid. *Chem Phys Lipids* 126: 1–27, 2003.
84. **Talpalar AE, Grossman Y.** CNS manifestations of HPNS: revisited. *Undersea Hyperb Med* 33: 205–210, 2006.
85. **Talpalar AE, Grossman Y.** Enhanced excitability compensates for high-pressure-induced depression of cortical inputs to the hippocampus. *J Neurophysiolo* 92: 3309–3319, 2004.
86. **Tank DW, Wu ES, Webb WW.** Enhanced molecular diffusibility in muscle membrane blebs: release of lateral constraints. *J Cell Biol* 92: 207–212, 1982.
87. **Theron M, Guerrero F, Sebert P.** Improvement in the efficiency of oxidative phosphorylation in the freshwater eel acclimated to 10.1 MPa hydrostatic pressure. *J Exp Biol* 203: 3019–3023, 2000.
88. **Thom SR.** Oxidative stress is fundamental to hyperbaric oxygen therapy. *J Appl Physiol (* October 9, 2008). doi:10.1152/japplphysiol.91004.2008.
89. **Thom SR, Bhopale VM, Mancini DJ, Milovanova TN.** Actin S-nitrosylation inhibits neutrophil beta2 integrin function. *J Biol Chem* 283: 10822–10834, 2008.
90. **Thom SR, Bhopale VM, Velazquez OC, Goldstein LJ, Thom LH, Buerk DG.** Stem cell mobilization by hyperbaric oxygen. *Am J Physiol Heart Circ Physiol* 290: H1378–H1386, 2006.
91. **Thom SR, Mendiguren I, Hardy K, Bolotin T, Fisher D, Nebolon M, Kilpatrick L.** Inhibition of human neutrophil β2-integrin-dependent adherence by hyperbaric O_2. *Am J Physiol Cell Physiol* 272: C770–C777, 1997.
92. **Trudell JR, Koblin DD, Eger IIEI.** A molecular description of how nobel gases and nitrogen bind to a model site of anesthetic action. *Anesth Analg* 87: 411–418, 1998.
93. **Urano S, Sato Y, Otonari T, Makabe S, Suzuki S, Ogata M, Endo T.** Aging and oxidative stress in neurodegeneration. *Biofactors* 7: 103–112, 1998.
94. **Valentine RC, Valentine DL.** Omega-3 fatty acids in cellular membranes: a unified concept. *Prog Lipid Res* 43: 383–402, 2004.
95. **van Meer G, Voelker DR, Feigenson GW.** Membrane lipids: where they are and how they behave. *Nat Rev Mol Cell Biol* 9: 112–124, 2008.
96. **Vettier A, Labbe C, Amerand A, Da Costa G, Le Rumeur E, Moisan C, Sebert P.** Hydrostatic pressure effects on eel mitochondrial functioning and membrane fluidity. *Undersea Hyperb Med* 33: 149–156, 2006.
97. **Wang X, Quinn PJ.** Vitamin E and its function in membranes. *Prog Lipid Res* 38: 309–336, 1999.
98. **Ward JPT.** Oxygen sensors in context. *Biochim Biophys Acta* 1777: 1–14, 2008.
99. **Webster NR, Toothill C.** Inorganic phosphate transport across the red blood cell membrane: the effect of exposure to hyperoxia. *Clin Chim Acta* 167: 259–265, 1987.
100. **White IL, Franks NP, Dickinson R.** Effects of isoflurane and xenon on Ba^{2+}-currents mediated by N-type calcium channels. *Br J Anaesth* 94: 784–790, 2005.
101. **Whittemore ER, Loo DT, Watt JA, Cotman CW.** A detailed analysis of hydrogen peroxide-induced cell death in primary neuronal culture. *Neuroscience* 67: 921–932, 1995.
102. **Wlodarczyk A, McMillan PF, Greenfield SA.** High pressure effects in anaesthesia and narcosis. *Chem Soc Rev* 35: 890–898, 2006.
103. **Yang B, Oo TN, Rizzo V.** Lipid rafts mediate H_2O_2 prosurvival effects in cultured endothelial cells. *FASEB J* 20: 1501–1503, 2006.