Current Thoughts on Mechanisms of Hyperoxic Seizures

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Hyperoxic seizures, like the weather, are a less that totally predictable element of diving. However, in the past five to ten years, a significant amount of knowledge has emerged regarding mechanisms of hyperoxic seizures. In this article, I'll review the current state of knowledge with regard to mechanisms of hyperoxic seizures, and offer some insights into how hyperoxic seizures can be precipitated by stress, drugs, and carbon dioxide (CO_2) retention. Our understanding of hyperoxic seizures is imperfect at best, and some of the information that I'll provide is conjecture based on known mechanisms. Possessing greater knowledge can make us safer divers, but if you're reading this hoping to find an easy method to protect yourself against hyperoxic seizures, you should stop. Our best defense against hyperoxic seizures, as always, is common sense. So for the less interested or enduring, you only need to know the partial pressure of oxygen in the gas mix you're breathing at your current depth. But for those who want to know a bit more, read on.

In recent years, studies of hyperoxic seizure mechanisms have turned from neurotransmitters to reactive oxygen species (ROS). Although commonly termed oxygen radicals or free radicals, not all ROS are radicals. In fact, one ROS that appears to play a key role in hyperoxic seizures is not a radical. Traditionally, ROS have been regarded as toxic by-products of cellular oxidative metabolism, and have been blamed for a number of processes, including aging. However, this is likely to be an incomplete story, as ROS do have beneficial functions including defense against infection. Additionally, recent evidence suggests that very low concentrations of ROS act as cellular messengers, participating in signaling which translates extracellular events into intracellular responses (24, 31, 33). This signaling effect is highly specific and unrelated to toxic effects of ROS. However, before I delve into the details of how

ROS are involved in hyperoxic seizures, I first need to cover some basic information including what ROS are, how they are formed and what roles they may play in cellular function.

Atoms and molecules are composed of a nucleus of neutrons (uncharged particles) and protons (positively charged particles) and electrons (negatively charged particles) which orbit the nucleus. Electrons are segregated into a number of orbitals, each of which is normally occupied by two electrons. When an orbital is occupied by only one electron, a radical is formed. Thus, radicals are molecules that have an unpaired electron. Because the energy gradient promotes formation of electron orbitals occupied by two electrons, radicals are generally highly reactive, acquiring electrons to complete their orbitals. A radical will remove an electron from a nearby molecule, converting the radical to a stable molecule, and usually forming another radical (which had the electron removed from it). Under some conditions, this is how radical "chain reactions" are initiated, producing a propagated series of reactions with cell molecules, usually lipids in the cell membrane. Radicals are sometimes termed "free radicals" because they exist free from other molecules and can move within the interior of the cell. However, because of the generally reactive nature of radicals, the time span that a "free" radical exists is usually very transient (fractions of a second), making study of radicals difficult.

All oxygen centered radicals are ROS; however, not all ROS are radicals. One product of radical chemistry is hydrogen peroxide (H_2O_2), which is a ROS but is not a radical, because all of its electrons are paired (i.e., it has no single electrons in orbitals). Hydrogen peroxide is toxic (and works as a disinfectant) because of the reactive nature of the oxygen. However, in the absence of other atoms or molecules to react with, hydrogen peroxide is relatively stable (which is why you can keep it in a bottle). Hydrogen peroxide is also uncharged (all of the positive and

negative charges are balanced), and can diffuse out of cells, potentially reacting with nearby cells. Other ROS are charged, and cannot leave the cell where they are produced.

Cells consume oxygen to produce adenosine triphosphate (ATP), a storage form of energy. During the production of ATP by mitochondria in cells, oxygen (O_2) undergoes a four electron reduction (gains four electrons) to two molecules of water (H_2O). Mitochondria are subcellular organelles that are specialized to produce energy for cells. Electrons are transferred to oxygen by cytochrome enzymes contained in the membrane of mitochondria. However, it appears that occasionally electrons escape during this transfer process, and can combine with free molecular oxygen (O_2) to form a superoxide radical, $O_2^{\bullet -}$. Because oxygen has gained one electron (e^-), it now has an unpaired electron, and is thus a radical, superoxide. Superoxide also has a negative charge from the electron in gained. Generation of superoxide is an unavoidable consequence of oxidative metabolism, and cells have developed defense systems to convert superoxide to less toxic species. A common fate for superoxide is to be converted to hydrogen peroxide and water by the enzyme superoxide dismutase (SOD). SOD is an important defense mechanism against superoxide, and three different forms of SOD exist in association with cells. Superoxide can also be converted into more toxic radicals, but such radicals do not appear to play a role in hyperoxic seizures. Although hydrogen peroxide is not a radical, it is reactive and can be toxic to cells. In brain, two enzymes, catalase and glutathione peroxidase, can detoxify hydrogen peroxide, but glutathione peroxidase appears to be relatively more important than catalase. To function, however, glutathione peroxidase requires another compound, reduced glutathione (GSH), which may become depleted during oxygen stress. Glutathione peroxidase uses reduced glutathione to react with hydrogen peroxide, forming water and oxidized

glutathione (GSSG). Thus, in brain, the major mechanism that detoxifies superoxide requires at least two enzymes, SOD and glutathione peroxidase, as well as reduced glutathione.

By now, I'm sure that you're wondering where hyperoxic seizures fit into this picture. Much past investigation into mechanisms of hyperoxic seizures focused on brain levels of gamma-aminobutyric acid (GABA) (36, 37). GABA is the primary inhibitory neurotransmitter in brain, and is responsible for limiting neural activity. During hyperoxia, brain GABA decreases, and investigators proposed that the loss of inhibition of neurotransmission led to seizures. However, later studies demonstrated that although brain GABA decreases during hyperoxia, seizures are not an inevitable consequence of this change (3, 41). The decrease in brain GABA is not consistent in all animals, and some species have hyperoxic seizures without change in brain GABA (27). Furthermore, drugs that elevate brain GABA are not protective against hyperoxic seizures (3, 17). Thus, data suggest that brain GABA concentration is unrelated to hyperoxic seizures.

ROS were first implicated in the 1970's, when it was observed that administration of reduced glutathione increased the time to seizure during hyperbaric oxygen exposure (13, 32). Although it was not understood at the time, today we know that this finding suggests that increased brain levels of hydrogen peroxide could be important in triggering hyperoxic seizures. Later studies have confirmed the protective effect of reduced glutathione (22). Although not studied in brain, studies in lung suggest that increased leakage of electrons from cytochrome oxidase enzymes is responsible for increased formation of ROS during hyperbaric oxygen (21). In brain, hydrogen peroxide concentration increases by 200% to 700% during exposure to 3 ATA O_2 (38, 39). Reduced glutathione may become depleted in brain during hyperoxia, impairing the brain's ability to detoxify hydrogen peroxide. Increasing brain concentration of reduced

glutathione likely provides more substrate for glutathione peroxidase to detoxify hydrogen peroxide. This data suggest that brain hydrogen peroxide increases significantly during hyperoxia, and that increasing the availability of reduced glutathione increases the time to seizure by reducing brain hydrogen peroxide concentration.

Further insights into ROS mechanisms of hyperoxic seizures are available from studies that altered brain levels of SOD and catalase. Mice genetically manipulated to overexpress SOD (transgenic animals which have extra copies of a SOD gene) are actually more likely to have hyperoxic seizures (29). Although this might seem unexpected, as reducing superoxide levels should be beneficial, SOD converts superoxide to hydrogen peroxide, which may then precipitate a seizure. Furthermore, drugs that inhibit SOD delay the onset of hyperoxic seizures in animals, likely by reducing SOD-dependent hydrogen peroxide production in brain (16, 29). Conversely, inhibition of brain catalase activity enhances susceptibility to hyperoxic seizures, likely by allowing hydrogen peroxide to accumulate (40). Finally, increasing brain levels of both SOD and catalase increases the resistance to hyperoxic seizures (39). This data strengthens the idea that hydrogen peroxide is an important trigger of hyperoxic seizures. This data also suggest hydrogen peroxide produced by SOD may indirectly contribute to a hyperoxic seizure.

Under normal conditions, brain enzymes other than SOD may produce hydrogen peroxide. One such enzyme is monoamine oxidase (MAO), which breaks down catecholamines (epinephrine, and norepinephrine/adrenaline and noradrenaline) to produce hydrogen peroxide. Drugs that inhibit MAO delay the onset of hyperoxic seizures, which suggests that hydrogen peroxide produced by MAO contributes to hyperoxic seizures (10, 40, 41). Although hydrogen peroxide may be produced in brain by both SOD and MAO, study of the relative importance of these two sources suggest that hydrogen peroxide from MAO is the more important source in

hyperoxic seizures (40). This suggests that elevation of catecholamine levels in brain could lead to increased hydrogen peroxide production, which could then cause a hyperoxic seizure. The importance of brain catecholamines in hyperoxic seizures can bee seen during normal changes in brain catecholamines. Catecholamines in brain have circadian variation, and are lowest during the early phase of the sleep cycle. Not surprisingly, the greatest tolerance to hyperoxia (longest time to seizure) occurs early in the sleep cycle of animals, when brain catecholamines are lowest (19). Without going into too much detail, the reader should understand that changes in blood catecholamine levels may not affect levels in brain because the blood-brain barrier prevents blood catecholamines from entering the brain. The brain has a separate system, which controls catecholamine levels in brain.

Hypercapnia has long been known to accelerate the onset of hyperoxic seizures. This effect has been thought to occur due to hypercapnia-mediated increased brain blood flow, resulting in increased brain O_2. This explanation may be overly simplistic, however. Carbon dioxide is a potent dilator of blood vessels in brain, and some studies suggest that increased CO_2 resulted in increased brain O_2 (5, 18, 26). However, other studies have reported that increasing CO_2 does not elevate brain O_2 (8, 20). Thus, it is not clear that increased CO_2 actually increases brain O_2, and other mechanisms may explain how CO_2 accelerates the onset of hyperoxic seizures.

During exposure to hyperbaric oxygen (without elevation of CO_2), brain O_2 initially rises to very high levels, and then quickly falls to lower levels (34). If CO_2 is then elevated during hyperbaric oxygen exposure, brain O_2 increases, but then within minutes falls to a lower level despite continued elevation of CO_2 (4). When brain O_2 is measured during hyperbaric oxygen exposure, seizures do not occur when brain O_2 is at peak levels, but typically occur when brain O_2

has fallen to less than half of the peak value (although brain O_2 remains above the value determined during air breathing) (34). Furthermore, during treatment in humans in hyperbaric chambers, seizures frequently occur during air breaks, a phenomenon that has been termed the "off effect", meaning that the seizure occurs when the patient is off oxygen. Data cited below regarding changes in brain O_2 and CO_2 during changes in breathing gas density support the concept that elevation of CO_2 alone may cause hyperoxic seizures. An alternative explanation for CO_2 precipitating a hyperoxic seizure is that elevation of CO_2 activates the sympathetic nervous system in brain, releasing catecholamines. This idea is supported by a study in isolated brain tissue, where elevation of CO_2 increases release of catecholamines from neurons (15). Thus, data support the idea that elevation of CO_2 may precipitate a seizure by releasing brain catecholamines, which are then broken down by MAO, increasing brain hydrogen peroxide, leading to a seizure.

Mechanisms by which hydrogen peroxide causes a seizure are not understood, but some information is available. Hydrogen peroxide is not particularly reactive, but can be reduced to hydroxyl radical ($OH^{\bullet}$), which is very reactive and toxic. Hydroxyl radical can react with cell lipids, forming lipid peroxides, and with sulfhydryl groups in proteins, forming disulfide bridges. Both of these reactions alter the function of cell lipid membranes as well as cell receptors and enzymes, which are composed of proteins. ROS-induced cell damage has been thought to result in seizures, and some data support a role for the toxic effects (as opposed to specific effects) of hydrogen peroxide in the genesis of hyperoxic seizures. In animals, administration of vitamin E (α-tocopherol) increases the time to hyperoxic seizures, and depletion of vitamin E reduces the time to seizure (23, 25, 42). Vitamin E is an antioxidant that resides in the lipid membrane of cells, and is thought to be important in terminating hydroxyl radical-initiated lipid peroxidation

chain reactions. Because vitamin E does not react with hydrogen peroxide, the protective effect of vitamin E could be mediated by reducing hydroxyl radical-induced peroxidation in cells. However, hyperoxic seizures can occur without measurable increase in brain lipid peroxides, which suggests that generation of hydroxyl radicals and cell lipid peroxides may not contribute to hyperoxic seizures (6). Furthermore, vitamin E supplementation elevates brain levels of reduced glutathione as well as reduces brain hydrogen peroxide concentration during hyperoxia (11, 23). Thus, vitamin E may indirectly lower brain hydrogen peroxide concentration by increased scavenging via reduced glutathione and glutathione peroxidase. Overall, it is not clear which effect of vitamin E is important in increasing the tolerance to hyperoxia, and it is possible that reduction of both hydroxyl radical and hydrogen peroxide contributes to the effect.

In contrast to toxic effects that damage cells, hydrogen peroxide may also exert highly specific effects on cells, independent of toxic damage. In isolated brain tissue, both hydrogen peroxide and superoxide cause release of excitatory amino acids (30). Elevated brain levels of excitatory amino acids causes seizures, and could explain how hydrogen peroxide initiates a seizure. Furthermore, in brain, hydrogen peroxide dilates blood vessels in a specific and reversible fashion, possibly increasing brain O_2, contributing to a seizure (35). Thus, there appear to be at least two specific effects of hydrogen peroxide, unrelated to toxic effects, which may contribute to the genesis of hyperoxic seizures.

Some drugs may elevate brain catecholamines, and thus predispose to hyperoxic seizures. A common decongestant is pseudoephedrine, which exerts its decongestant effect by causing release of catecholamines outside of the brain (2). Pseudoephedrine can also produce symptoms of central nervous system stimulation, which likely results from elevation of catecholamines in brain. Although not studied, it is possible that pseudoephedrine may contribute to hyperoxic

seizures by elevating brain catecholamines, which are then metabolized by MAO, yielding hydrogen peroxide. Phenylpropanolamine is another decongestant that functions much like pseudoephedrine, and may likely have a similar effect of exacerbating hyperoxic seizures (1).

Caffeine is a central nervous system stimulant that, under some conditions, elevates brain catecholamines (28). Very high doses of caffeine alone can cause seizures. However, it is likely that amounts of caffeine consumed by humans does not significantly alter brain catecholamines. The effects of caffeine on brain are complex, and caffeine interacts with a number of systems in brain other than catecholamines (28). Furthermore, caffeine constricts cerebral blood vessels and reduces cerebral blood flow (28), and thus may reduce oxygen tension in brain during hyperoxia. In spite of potential changes in brain catecholamines, when caffeine was tested in rats, it delayed the onset of hyperoxic seizures (9). How caffeine delays the onset of hyperoxic seizures is not known, and points out that however caffeine stimulates the brain, it is not in a fashion which exacerbates hyperoxic seizures. Caffeine has been reported to act as an antioxidant, but this effect requires caffeine levels that are far above levels which occur after consumption of caffeinated beverages (14).

There are several instructive points to be learned from the above information. First, most of the studies cited were conducted in animals and were not performed to develop a method to protect against hyperoxic seizures, but rather to understand mechanisms that trigger such seizures. Clearly, some of the interventions are not possible in humans (transgenes) and others may have toxic side-effects. More importantly, though, all of the treatments only delayed, usually by minutes, and did not prevent the onset of hyperoxic seizures. So hyperoxic seizures cannot be completely eliminated, and in humans it is not likely that any drug treatment would reliably increase the tolerance to hyperoxia. Secondly, and more importantly, there are some

circumstances which can reduce tolerance to hyperoxia, making a hyperoxic seizure more likely. Understanding these circumstances and avoiding them may provide some additional safety during in water exposure to elevated PO_2. The genesis of a hyperoxic seizure is likely to be multifactoral, with several factors contributing to the seizure. The reader should note that much of what follows is conjecture based on the knowledge presented above. Elevation of brain catecholamines seems to be a key element in the genesis of hyperoxic seizures. Stress, both mental and physical, activates the sympathetic nervous system in the brain and elsewhere in the body, releasing catecholamines and potentially contributing to a seizure. Stress factors that have been shown to exacerbate hyperoxic seizures include thermal stress (hypothermia) and physical stress (exertion). Some stress in diving is unavoidable, but stress due to illness, fatigue or lack of sleep is avoidable. Furthermore, dehydration can activate the sympathetic nervous system, and could contribute to elevating brain catecholamine levels. As always, divers should avoid dehydration and be aware of their stress level both before and during a dive. Reduction of PO_2 exposure during high exertion dives or in cold water could be wise, particularly when several exacerbating factors are combined.

Avoiding CO_2 elevation is also a key step to avoiding a hyperoxic seizure. Some individuals are predisposed to retain CO_2 during diving, although the factors that contribute to CO_2 retention in individuals is not clear (12). Poor regulator performance or restrictive suits may lead to CO_2 retention, but other less obvious factors may also elevate CO_2. Gas density has an important influence on the efficiency of respiratory exchange, and as gas density increases, CO_2 increases (7, 8). Gas density increases as the molecular weight of the gas increases, or as depth increases. Increased gas density may elevate CO_2 by reducing the efficiency of respiratory exchange secondary to increased turbulent gas flow in the airway. Under experimental

conditions, brain CO_2 is higher and O_2 is lower in animals breathing hyperbaric nitrogen/oxygen as compared to helium/oxygen, and the time to seizure is shorter with nitrogen/oxygen (8). This data suggest that the elevation of CO_2 alone is important in precipitating the seizure, as brain O_2 was actually lower during nitrogen/oxygen breathing. Humans also have higher CO_2 levels when breathing nitrogen/oxygen as opposed to helium/oxygen mixtures (12). This data suggest that switching from a less dense gas (trimix, heliox) to a more dense gas (air) at depth would likely elevate CO_2, which may predispose a diver to a hyperoxic seizure.

After wading through all of this information, you should wonder "What is the practical take-home message from this?" A key issue in precipitating a hyperoxic seizure appears to be elevation of brain catecholamines, which may occur with stress, elevation of CO_2, and decongestants. Avoidance of circumstances leading to elevated brain catecholamines may provide some addition safety during in water exposure to maximal PO_2's. Divers should be continually aware of their stress level, both before and during a dive. Divers should also consider that stress factors may be unavoidable in a dive, including cold water exposure and high exertion. Proper equipment maintenance is critical, as decremental reduction in regulator performance can lead to CO_2 retention, and may contribute to a hyperoxic seizure. Finally, avoidance of dehydration and decongestants is likely to be beneficial in avoiding hyperoxic seizures.

GLOSSARY

Catalase: An enzyme that detoxifies hydrogen peroxide by converting it to water and oxygen.

Catecholamine: A natural or synthetic compound that activates adrenergic receptors in the body. Examples include epinephrine (adrenaline) and norepinephrine (noradrenaline). Catecholamines are released by the sympathetic nervous system.

Dismutation: The conversion of two identical molecules to two dissimilar molecules. SOD dismutates two molecules of superoxide radical to one molecule each of oxygen and hydrogen peroxide.

Hydrogen peroxide (H_2O_2): A reactive oxygen species form by the two electron reduction of molecular oxygen (O_2).

Hypercarbia or hypercapnia: Elevation of carbon dioxide (CO_2) partial pressure above normal. May occur due to reduction in ventilation, increased breathing gas density, poor regulator performance or constrictive suits.

Hyperoxia: Elevation of oxygen (O_2) partial pressure above normal.

Hypocarbia or hypocapnia: Reduction of carbon dioxide (CO_2) partial pressure below normal. Usually occurs due to increased ventilation.

Monoamine oxidase (MAO): An enzyme that breaks down catecholamines to release hydrogen peroxide.

Oxidation: The loss of an electron from an atom or molecule. During dismutation of two superoxide radicals, one radical is reduced to hydrogen peroxide (it gains an electron), and the other is oxidized to oxygen (it loses an electron).

Radical: An atom or molecule that has an unpaired electron.

Reactive oxygen species (ROS): Molecules that contain partially reduced oxygen. A one electron reduction of molecular oxygen (O_2) yields superoxide, two electron reduction of oxygen yields hydrogen peroxide, three electron reduction of oxygen yields hyroxyl radical and four electron reduction of oxygen yields water, which is the fully reduced form of oxygen.

Reduction: The addition of an electron to an atom or molecule.

Superoxide dismutase (SOD): A family of enzymes that converts superoxide radicals to hydrogen peroxide and water.

Sympathetic nervous system: The part of the nervous system that releases catecholamines, usually in response to stress. Activation of the sympathetic nervous system is important in the "fight or flight" response.

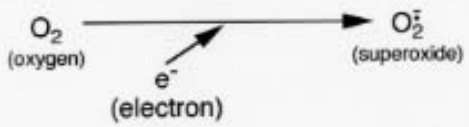
A Note on Reactive Oxygen Species Symbology

The symbology attached to the chemical formulas of ROS has specific meanings. A dot ($^{\bullet}$) next to the atom or molecule denotes an unpaired electron, which makes the atom or molecule a radical. A minus sign ($^{-}$) denotes a negative charge, meaning that there is one more negative charge than positive charges in the atom or molecule. Some ROS such as superoxide ($O_2^{\bullet -}$) have both an unpaired electron and a negative charge, as denoted by the symbols, and others like hydrogen peroxide (H_2O_2) has neither a unpaired electron or charge, so it has neither symbol.

Figure 1: Reactive Oxygen Species Chemistry

A. Generation of superoxide

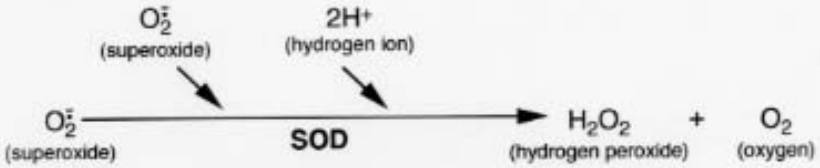
Spontaneous from electron leak in mitachondria



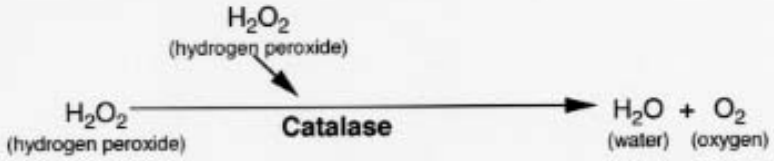
Generation by enzymes

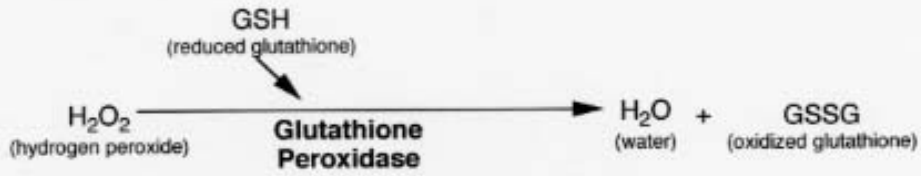
NADPH Diaphorase
Xanthine Oxidase
Cyclooxygenase

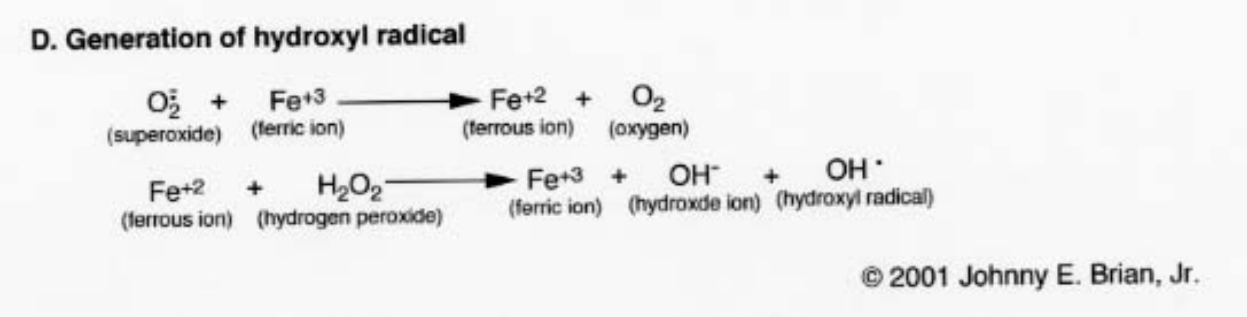
B. Generation of hydrogen peroxide



C. Scavenging of hydrogen peroxide

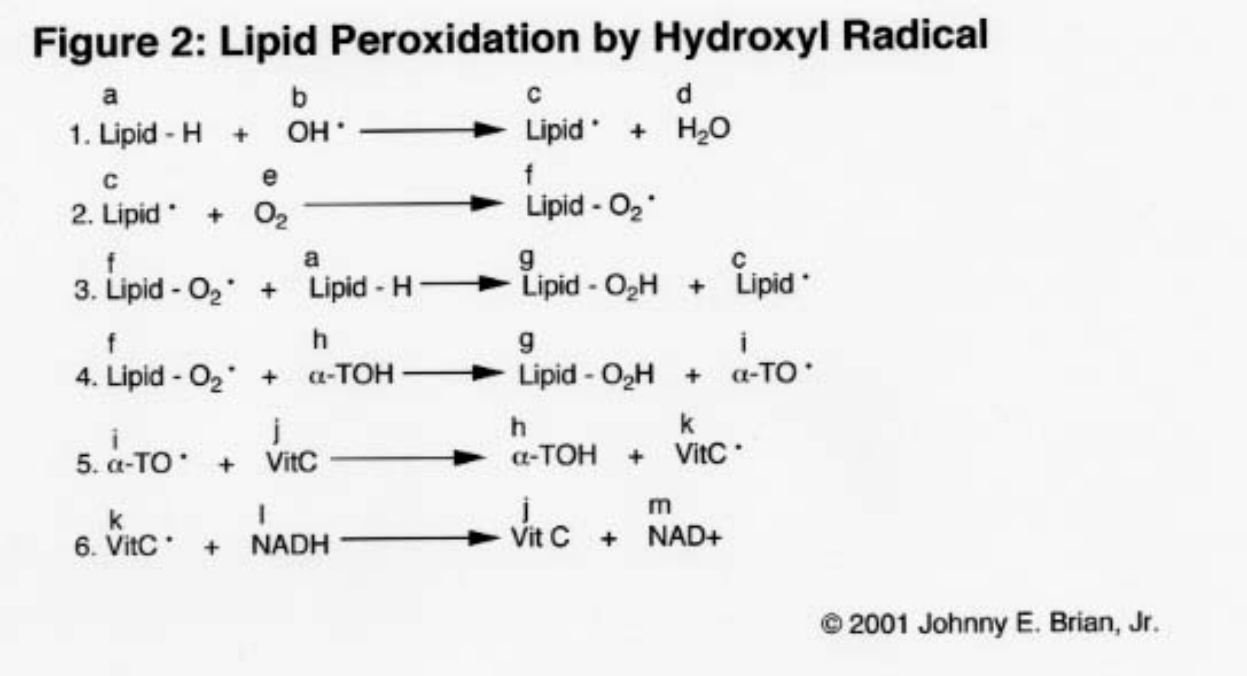






Legend – Figure 1

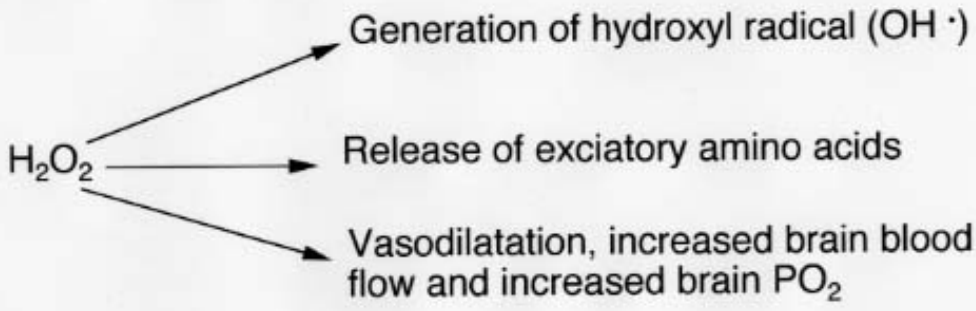
A. Superoxide can be generated spontaneously when molecular oxygen acquires an electron, most likely from leakage associated with electron transport in mitochondria, or by a number of intracellular enzymes that can generate superoxide. During hyperoxia, leakage of electrons from mitochondria increases, and likely increases formation of superoxide. B. Hydrogen peroxide is formed when superoxide is dismutated into hydrogen peroxide and oxygen by the enzyme superoxide dismutase (SOD). Hydrogen peroxide is also formed when catecholamines are metabolized by the enzyme monoamine oxidase (MAO). During hyperoxia, both sources contribute to formation of hydrogen peroxide, but MAO appears to be relatively more important. C. Hydrogen peroxide is scavenged by two enzyme systems, catalase that converts hydrogen peroxide to water and oxygen, and glutathione peroxidase that requires reduced glutathione as a co-factor, converting hydrogen peroxide to water and oxidized glutathione. D. Hydroxyl radicals are generated when superoxide transfers an electron to a ferric ion, producing oxygen and a ferrous ion. The ferrous ion can then react with hydrogen peroxide, regenerating the ferric ion and producing a hydroxyl radical and a hydroxide ion. Hydroxyl radicals are very reactive and cause cellular damage associated with reactive oxygen species.



Legend – Figure 2

In reaction 1, a lipid (a) reacts with a hydroxyl radical (b), forming a lipid radical (c) and water (d). In reaction 2, the lipid radical (c), reacts with oxygen (e), forming a lipid peroxyl radical (f). In reaction 3, the lipid peroxyl radical (f) reacts with another lipid (a), forming a stable, nonradical lipid hydroperoxide (g) and another lipid radical (c). Although the lipid hydroperoxide is stable, it represents a permanent alteration in the structure of the lipid. The lipid radical formed in reaction 3 can then re-enter reaction 2, thus propagating a chain reaction. In reaction 4, the lipid peroxy radical (f) reacts with α-tocopherol (h; vitamin E), forming a lipid hydroperoxide (g) and a α-tocopherol radical (i), which has relatively low reactivity. In reaction 5, the α-tocopherol radical (i) reacts with vitamin C (j), regenerating α-tocopherol (h) and a vitamin C radical (k). Vitamin C is regenerated in reaction 6 by NADH (l).

Figure 3: Mechanisms of Hyperoxic Seizures



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