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Reactive Oxygen Species and the Brain in Sleep Apnea

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Abstract

Rodents exposed to intermittent hypoxia (IH), a model of obstructive sleep apnea (OSA), manifest impaired learning and memory and somnolence. Increased levels of reactive oxygen species (ROS), oxidative tissue damage, and apoptotic neuronal cell death are associated with the presence of IH-induced CNS dysfunction. Furthermore, treatment with antioxidants or overexpression of antioxidant enzymes is neuroprotective during IH. These findings mimic clinical cases of OSA and suggest that ROS may play a key causal role in OSA-induced neuropathology. Controlled production of ROS occurs in multiple subcellular compartments of normal cells and de-regulation of such processes may result in excessive ROS production. The mitochondrial electron transport chain, especially complexes I and III, and the NADPH oxidase in the cellular membrane are the two main sources of ROS in brain cells, although other systems, including xanthine oxidase, phospholipase A2, lipoxygenase, cyclooxygenase, and cytochrome P450, may all play a role. The initial evidence for NADPH oxidase and mitochondrial involvement in IH-induced ROS production and neuronal injury unquestionably warrants future research efforts.

1. Introduction

Reactive oxygen species (ROS) can be generated from various subcellular compartments, including mitochondria, the cellular membrane, lysosomes, peroxisomes, and the endoplasmic reticulum (Angermuller et al., 2009; Bedard and Krause, 2007; Droge, 2002; Kubota et al., 2010; Santos et al., 2009). While ROS production in mitochondria relies solely on the electron transport chain, it usually involves multiple enzymatic systems in other subcellular compartments. For example, NADPH oxidase (Akki et al., 2009; Bedard and Krause, 2007), xanthine oxidase (Berry and Hare, 2004), phospholipase A2 (Muralikrishna Adibhatla and Hatcher, 2006), lipoxygenases and cyclooxygenase (Droge, 2002), and cytochrome P450 (Yasui et al., 2005) have all been identified as sources of ROS in various subcellular compartments under both physiological and pathological conditions (Figure 1). However, since mitochondria and NADPH oxidase are arguably the predominant sources of ROS in the central nervous system and have been more recently shown to play a role in intermittent hypoxia-induced neuronal deficits, the current review will focus on these two systems and their interactions. Involvement of other ROS-producing systems in sleep apnea-related neuropathology has not been thus far either explored or confirmed. However, such involvement should not be excluded and definitely warrants additional future investigation.

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2. Mitochondria

Mitochondria are the major cellular source of reactive oxygen species (ROS) in most non-phagocytic cells under normal conditions. As the cellular power plant, mitochondria convert energy contained in nutrients to ATP, the universal energy currency of all biological systems, through oxidative phosphorylation. During this process, a pair of electrons is donated by NADH to complex I (NADH-ubiquinone oxidoreductase) or by FADH₂ to complex II (succinate dehydrogenase) of the electron transport chain (ETC) in the inner mitochondrial membrane. The electrons are then passed along the ETC in the order of complex I → III → IV or II → III → IV and are accepted by molecular oxygen at complex IV (cytochrome c oxidase) through 4-electron reduction of oxygen, generating water (Berg et al., 2002). When electrons flow down the complexes, energy is released and used to translocate protons from the mitochondrial matrix to the intermembrane space, creating a proton gradient across the inner membrane, also known as the mitochondrial membrane potential (Schultz and Chan, 2001). Energy stored in this gradient is then used to synthesize ATP from ADP in a phosphorylation reaction, when protons flow back to the matrix across the inner membrane through ATP synthase (Boyer, 1997). The efficiency of electron transport, however, is less than 100% and some electrons “leak” from the flow at various locations in the ETC (see below), resulting in one-electron reduction of oxygen, generating superoxide (O₂^{•−}), even under physiological conditions (Halliwell, 2006). Superoxide is usually readily converted to hydrogen peroxide (H₂O₂) by manganese superoxide dismutase (MnSOD) in the matrix or Copper/Zinc SOD (Cu/ZnSOD) in the intermembrane space (Halliwell, 2006; Turrens, 2003). Although hydrogen peroxide is further reduced to water by peroxiredoxins, glutathione peroxidase, or, in some organs, catalase (Andreyev et al., 2005), it may also react with reduced transition metal ions, such as Fe²⁺, to form the hydroxyl radical (•OH), especially when there is an imbalance between its production and the capacity of antioxidants that reduce it as specified above. The hydroxyl radical is a highly reactive radical that causes peroxidation of all cellular constituents, including lipids, proteins, and nucleic acids, and is believed to be mainly responsible for oxidative stress-induced cellular damage (Oliver et al., 1990).

While a multiplicity of electron-leaking sites have been identified in mitochondria (Andreyev et al., 2005; Brand, 2010), the sites with the greatest maximum capacities to produce superoxide reside within complex I and complex III (ubiquinol-cytochrome c oxidoreductase) (Figure 2) (Brand, 2010; Murphy, 2009). Complex I may produce superoxide via two distinct mechanisms. In isolated mitochondria supported by NADH-producing substrates, such as pyruvate plus malate, electrons are passed from the flavin mononucleotide (FMN) site through the Fe–S clusters to ubiquinone in the multi-subunit complex I before reaching complex III. This forward electron transport produces superoxide at a moderate rate during normal respiration, which increases by several-fold in the presence of rotenone (Gyulkhandanyan and Pennefather, 2004; Kushnareva et al., 2002). As rotenone blocks electron transport near the ubiquinone binding site, it presumably causes electrons to back up and to reduce the upstream redox components, namely the Fe–S clusters and the FMN site, resulting in electron leakage at these sites. There is evidence suggesting electron leakage towards the matrix at both the FMN site (Johnson et al., 2003; Kudin et al., 2004) and the Fe–S clusters (Kushnareva et al., 2002). The other mechanism by which complex I produces large amounts of superoxide is during reverse electron transport, which occurs within complex I when mitochondria are supported by FADH₂-producing substrates, such as succinate (Hinkle et al., 1967). These substrates reduce ubiquinone and generate a proton motive force, driving electrons thermodynamically uphill through complex I to reduce NAD⁺ to NADH at the FMN site and to produce superoxide at a rate considerably higher than that through the forward electron transport (Gyulkhandanyan and Pennefather, 2004; Kamba et al., 2010). Although the site of electron leakage during reverse electron transport remains

to be ascertained, evidence suggests that it may be at or near the ubiquinone-binding site (Lambeth, 2004). The fact that rotenone effectively blocks superoxide production in succinate-supported mitochondria is consistent with this notion. Several studies have shown that superoxide production through reverse electron transport in complex I in isolated mitochondria is dependent upon the presence of a high proton gradient across the inner membrane (Liu et al., 2002; Votyakova and Reynolds, 2001). As with forward electron transport, leakage of electron through reverse electron transport is also towards the matrix. It is important to point out that the true biological significance of each of the electron-leaking sites in complex I, especially those involved in superoxide production through reverse electron transport, is not fully understood at present (Brand, 2010; Murphy, 2009). This is because much of the literature has been based on studies using isolated mitochondria or submitochondrial particles oxidizing either NADH- or FADH₂-producing substrates during state 4 respiration, i.e., when no ATP is generated. These conditions are certainly different from those in mitochondria in living cells where multiple substrates are available and producing both NADH and FADH₂ at various ratios and, importantly, the proton gradient is used to generate ATP, which facilitates electron transport. Some of the more recent studies have attempted to address the significance of the high-rate superoxide production through reverse electron transport in living cells. While some found that rotenone decreased cellular ROS levels, supporting a significant role for ROS production in complex I through reverse electron transport (Aon et al., 2003; Vrablic et al., 2001); others found just the opposite, suggesting that ROS production in complex I through the forward electron transport might be more important (Li et al., 2003; Nakamura et al., 2001). It is possible that these results, relying on the use of rotenone, might be influenced by the metabolic state of the cells, especially the predominant endogenous substrate(s) that was used by those cells. Additional studies using independent methods are needed to further clarify this issue.

Complex III passes electrons from ubiquinol to cytochrome c through cyclic oxidation and reduction of the electron carrier, ubiquinol-ubiquinone, at its Q_o (located near the outer surface of the inner membrane) and Q_i (located near the inner surface of the inner membrane) centers, respectively. This process is known as the “Q cycle”, during which four protons are translocated to the intermembrane space while two electrons are accepted by cytochrome c (Mitchell, 1975). Production of superoxide in complex III is well-established, and occurs mainly at the Q_o center (Han et al., 2003; Kudin et al., 2004; Muller et al., 2004; St-Pierre et al., 2002). High rates of superoxide production can occur in the presence of antimycin A. This Q_i center inhibitor blocks electron flow from the Q_o center to the Q_i center, causing a build-up of semiquinone at the Q_o center, which in turn leads to electron leakage and superoxide production (Cape et al., 2007). Q_o center inhibitors, such as myxothiazol and stigmatellin, stop electrons from accessing the center and prevent superoxide production (Turrens et al., 1985), supporting the notion that the main superoxide production site at complex III is indeed the semiquinone at the Q_o center. ROS produced at the Q_o center is thought to be released towards both the intermembrane space and the matrix (Han et al., 2003; Kudin et al., 2004; Muller et al., 2004; St-Pierre et al., 2002). There is also evidence suggesting the presence of superoxide production at the Q_i center (Raha et al., 2000), which would be released towards the matrix. Although the rate of superoxide production at complex III may be low in comparison to those at complex I, especially those through reverse electron transport, in normal mitochondria, its significance under certain pathological conditions have been recognized. Importantly, ROS produced at complex III have been shown to be critical in controlling HIF-1 α on and, in turn, HIF-2 α mediated transcriptional regulation in hypoxic cells (Bell et al., 2007; Guzy et al., 2005; Guzy and Schumacker, 2006). It is also implicated in ischemia/reperfusion injury (Chen et al., 2003; Chen et al., 2010).

While commonly regarded as obligatory “by-products” of ATP production through oxidative phosphorylation, ROS produced in mitochondria play indispensable roles in various cellular processes, including metabolic regulation (Fedotcheva et al., 2006), oxygen sensing (Guzy and Schumacker, 2006), and preconditioning (Kimura et al., 2005), and their production is actively and tightly regulated (Gutierrez et al., 2006; Matsuzaki et al., 2009; Stowe and Camara, 2009). It can be argued, therefore, whether ROS are merely “by-products” or in fact “primary products” by mitochondria. Under pathological conditions, functional disturbance and/or structural damage of mitochondria increase the rate of electron leakage at various locations (Chen et al., 2003; Gyulkhandanyan and Pennefather, 2004; Kamga et al., 2010; Khan et al., 2010) and ROS production from mitochondria becomes deregulated. The initial oxidative stress can be amplified, through weakening of mitochondrial/cellular antioxidant defenses (Kamga et al., 2010), activating other cellular sources of ROS (e.g., the NADPH oxidase; see below), and increasing mitochondrial membrane permeability (Brady et al., 2006; Zorov et al., 2006), and can result in additional ROS production and severe oxidative stress, which causes irreversible damage to mitochondria and eventually cell death. Indeed, oxidative stress-induced cellular injury and cell death have been implicated in a spectrum of pathological conditions, including aging (Page et al., 2010), stroke (Halliwell, 2006), and degenerative diseases (Lin and Beal, 2006).

ROS production by mitochondria can be regulated by factors that affect electron flow, such as mitochondrial membrane potential (Boveris and Chance, 1973; Korshunov et al., 1997) and electron transport inhibitors (e.g., rotenone and antimycin A), by ROS themselves in a “ROS-induced ROS release” manner (Brady et al., 2006; Zorov et al., 2006), by hyperoxia (Turrens et al., 1982), and by hypoxia/ischemia (Chandel et al., 1998; Chen et al., 2010; Guzy et al., 2005; Hoffman et al., 2007). The latter probably reflects the fact that intramitochondrial oxygen levels under those conditions remain higher than the K_m for oxygen of cytochrome *c* oxidase and electron transport, at least some, was preserved to supply electrons for superoxide production. The exact mechanism underlying hypoxia-induced ROS production in mitochondria is unclear at present (Murphy, 2009; Stowe and Camara, 2009).

3. NADPH oxidase

NADPH oxidase is a multi-subunit enzyme complex, localized in both the plasma membrane and membranes of subcellular organelles, that catalyzes electron transfer from NADPH to molecular oxygen, producing superoxide. NADPH oxidase was first identified in phagocytes where its ROS-producing function plays an essential role in non-specific host defense against microbes during phagocytosis (Lambeth, 2004). It was soon found that enzyme systems similar to the phagocyte NADPH oxidase existed in many other cell types and subsequently a total of seven NADPH oxidase isoforms have been identified, including NOX1-5 and DUOX1-2 (Bedard and Krause, 2007; Lambeth, 2004). These isoforms differ from each other in their catalytic cores and their requirements for regulatory subunits. They are, however, believed to share the same fundamental mechanism of action as characterized in the prototypic NADPH oxidase in phagocytes, known as NOX2 (Bedard and Krause, 2007).

NOX2 contains the transmembrane catalytic core gp91^{phox}, the small transmembrane subunit p22^{phox}, and four cytosolic regulatory subunits, namely p47^{phox}, p67^{phox}, p40^{phox} and Rac1 or Rac2 (Figure 3) (Bedard and Krause, 2007). The catalytic core gp91^{phox} contains binding sites for NADPH and FAD and two heme domains that can undergo cyclical reduction and oxidation. These structural features allow NOX2 to catalyze a transmembrane redox reaction in a two-step process, using NADPH as the substrate (Nisimoto et al., 1999). In the first step of the redox reaction, electrons donated by NADPH

at the cytosolic side are transferred to FAD. A single is then transferred to the iron center of the inner heme in the second step. electron from FADH₂ The electron is in turn transferred to the outer heme and eventually accepted by molecular oxygen, forming superoxide, at the outer side of the membrane (Nisimoto et al., 1999). This superoxide is reduced by extracellular and/or cytosolic SODs to H₂O₂, which can diffuse to both sides of the membrane and play either physiological or pathological roles in various cellular processes. Interestingly, under resting conditions the four cytosolic regulatory subunits are physically dissociated from the two membrane-bound subunits, the catalytic core gp91^{phox} and the small subunit p22^{phox}, and the NOX2 enzyme is inactive. Assembly of an active enzyme complex occurs when translocation of cytosolic regulatory subunits is triggered by stress signals or agonists (Groemping and Rittinger, 2005). During this process, the p47^{phox} subunit is first phosphorylated and docked onto the p22^{phox} subunit. The p47^{phox} subunit then acts to organize the translocation of other cytosolic subunits, hence the designation as the “organizer subunit” (El-Benna et al., 2009). Once the p67^{phox} subunit, known as the “activator subunit,” is in contact with the catalytic core gp91^{phox}, its activation domain may directly interact with the flavocytochrome and thereby participate in the regulation of electron flow from NADPH to FAD in gp91^{phox} (Figure 3) (Nisimoto et al., 1999). There is evidence suggesting that this subunit-assembly process is actively controlled and represents an important regulatory step in the overall activity of the NADPH oxidase system (Lee et al., 2006; Rathore et al., 2008). NADPH oxidase is also regulated at the gene expression level (Desouki et al., 2005; Wosniak et al., 2009) and possibly at the substrate availability level in some settings (Gupte, 2008). Although NADH may also serve as a substrate, its affinity with NADPH oxidase family members is much lower (Clark et al., 1987). In this regard, the activity of glucose-6-phosphate dehydrogenase could be important since this is a key determinant of NADPH levels in the cell (Gupte, 2008).

Expression of NOX2 has been detected in phagocytes as well as in many nonphagocytic cells, including cells in the central nervous system, such as neurons (Serrano et al., 2003; Tammariello et al., 2000; Tejada-Simon et al., 2005), microglia (Lavigne et al., 2001), and astrocytes (Abramov et al., 2004). NOX2-derived ROS in these cells have been implicated in a wide variety of physiological processes through their diverse downstream signaling pathways, including activation of redox-sensitive kinases, modification of redox-sensitive transcription factors, and direct effects on enzymes, ion channels, and receptors (Akki et al., 2009). In the central nervous system, NOX2-derived ROS are thought to play roles in long-term potentiation (Kamsler and Segal, 2004; Tejada-Simon et al., 2005) and cell growth (Ibi et al., 2006). On the other hand, de-regulated activation of NOX2 leads to ROS production in large quantities that are likely involved in oxidative cellular damage in many pathological conditions in the central nervous system. Examples include stroke (Kahles et al., 2007), thrombin-/IL-13-induced neurotoxicity (Park et al., 2009), lipopolysaccharide-induced neurotoxicity (Qin et al., 2004), and neurodegeneration including Alzheimer’s disease (Choi et al., 2005), Parkinson’s disease (Gao et al., 2003), and amyotrophic lateral sclerosis (Wu et al., 2006). Of particular interest, NADPH oxidase-induced ROS production and subsequent oxidative stress have been implicated in intermittent hypoxia, an experimental model for sleep apnea (Khan et al., 2010; MacFarlane et al., 2009; Zhan et al., 2005). Its involvement in other cellular responses to hypoxia has also been reported, including hypoxic pulmonary vasoconstriction (Dennis et al., 2009; Rathore et al., 2008), hypoxia-induced chemoreceptor hypersensitivity (He et al., 2010), and hypoxia-induced mobilization of endothelial progenitor cells (Schroder et al., 2009), among others. Upregulation of the NADPH oxidase system seems to involve at least HIF-1 α (Diebold et al., 2010; Malec et al., 2010).

4. Cross-talk between mitochondria and the NADPH oxidase

While mitochondria and the NADPH oxidase are each capable of producing superoxide independently, emerging evidence suggests the existence of a cross-talk between the two cellular systems in which they appear to be co-stimulatory (Daiber, 2010). Several studies have shown that mitochondria may regulate superoxide production by NADPH oxidase. For example, increased NADPH oxidase activity and superoxide production induced by hypoxia or serum withdrawal were diminished by inhibition of mitochondrial ROS generation with electron transport chain inhibitors (e.g., rotenone) (Lee et al., 2006; Rathore et al., 2008). Mitochondrial ROS-induced activation of phosphoinositide 3-kinase (PI3K) and protein kinase C epsilon (PKC ϵ) subsequent phosphorylation and membrane translocation of NADPH oxidase regulatory subunits (e.g., p47^{phox} and Rac1) are believed to be the key link between the two systems (Lee et al., 2006; Rathore et al., 2008); while regulation of NADPH oxidase gene expression by mitochondrial ROS and/or other mitochondrial signals may also play a role (Desouki et al., 2005; Wosniak et al., 2009). Conversely, the NADPH oxidase system may regulate ROS production by mitochondria. There has been convincing evidence showing that NADPH oxidase inhibition, by either pharmacological agents (e.g., apocynin) or gene knockout/gene silencing of NADPH oxidase subunits (e.g., gp91^{phox} and p22^{phox}) abolishes or substantially attenuates mitochondrial superoxide production induced by various stimuli, including angiotensin II (Doughan et al., 2008; Kimura et al., 2005), intermittent hypoxia (Khan et al., 2010), and β -amyloid peptides (Abramov et al., 2004). The opening of mitochondrial ATP-sensitive potassium channels (mtK_{ATP}) and the mitochondrial permeability transition pore (mPTP) seems to mediate this NADPH oxidase-to-mitochondria signaling process. In this process, NADPH oxidase-derived ROS change cytosolic redox, which in turn activates mtK_{ATP} and mPTP, leading to a collapse of mitochondrial membrane potential and release of ROS from mitochondria (Daiber, 2010). Indeed, 5-hydroxydecanoate (5-HD, an mtK_{ATP} blocker) or cyclosporine A (CsA, an mPTP inhibitor) diminishes mitochondrial production of superoxide triggered by various NADPH oxidase activators (Doughan et al., 2008; Kimura et al., 2005; Wenzel et al., 2008). Obviously, the existence of such a cross-talk mechanism between the NADPH oxidase and mitochondria as described above would allow the two systems to form a positive feedback loop that amplifies the original signal regardless of its origination. Thus, cross-talk between these two cardinal systems underlying ROS production may play an important role in the severe increase of cellular oxidative stress and eventual cellular injury in many disease conditions.

5. What is the evidence for ROS involvement in intermittent hypoxia and sleep apnea?

5.1. The Clinical Spectrum of SBD and OSAS

Before we address the main question, it seems appropriate to provide a quick overview of the clinical term of sleep-disordered breathing (SDB). Indeed, SDB encompasses a spectrum of respiratory disturbances during sleep, ranging from intermittent snoring to obstructive sleep apnea syndrome (OSAS). OSAS, the most severe form of SBD, affects approximately 3–5 % of the general population including children, and is characterized by repeated episodes of upper airway obstruction during sleep (Lumeng and Chervin, 2008; Punjabi, 2008). The intermittent upper airway obstruction during sleep characteristically leads to a spectrum of mild to severe intermittent hypoxia (IH) episodes, with nadir hemoglobin oxygen saturations potentially reaching the lower limits of oximeters (50–60%) (Figure 4). In addition, periodic alveolar hypoventilation and repeated behavioral and/or electroencephalographic arousals occur, leading to sleep fragmentation and deprivation.

SDB and OSAS in particular, are now recognized as being associated with substantial neuropsychological health problems, in addition to the serious cardiovascular and metabolic consequences of the disease (for comprehensive reviews see: Beebe, 2005; Bonsignore and Zito, 2008; Gozal and Kheirandish-Gozal, 2007, 2008). The major neuropsychological sequelae of OSAS in humans include excessive daytime sleepiness, personality and psychosocial maladjustment patterns, and mental impairment consistent with disordered function of the prefrontal cortex and/or medial temporal lobe (Beebe and Gozal, 2002). However, the relative contribution of the intermittent hypoxia and the sleep fragmentation/deprivation components to the neurocognitive deficits observed in OSAS patients is still unclear, necessitating the development of an appropriate animal model to explore the effects of IH on cognitive function (Sateia, 2003).

5.2. Intermittent Hypoxia and NADPH Oxidase

To further study the effects of IH on CNS, a rodent model was developed in our laboratory, whereby the neurobehavioral effects of episodic or intermittent hypoxia can be assessed in the absence of significant sleep fragmentation (Gozal et al., 2001a). Young adult male rats were exposed to an intermittent hypoxia profile designed to produce similar nadir hemoglobin oxygen saturations as observed in OSAS patients, consisting of alternating 90 second epochs of hypoxia (10% O₂) and room air during habitual sleep times, with minimal disruption of sleep architecture. Such models have since been extended to mice, and have allowed for initiation of efforts aiming to provide a more precise dissection of genetic mechanisms involved in the molecular and cellular adaptations and injury associated with IH (de Frutos et al., 2010; Fan et al., 2005; Jun et al., 2010; Kheirandish et al., 2005; Peng et al., 2009; Xu et al., 2009).

Behaviorally, exposure of Sprague-Dawley rats to IH during sleep slowed acquisition and impaired retention of a hippocampal-dependent learning task, a spatial reference version of the Morris water maze (Gozal et al., 2001a), but had no effect on sensorimotor function. The behavioral deficits were preceded by increased apoptosis in medial temporal lobe structures implicated in learning and memory, which displayed both a characteristic temporal and regional profile. Apoptosis in the hippocampal CA1 region and the frontoparietal cortex peaked at 1 and 2 days of IH, and decreased thereafter. Moreover, while apoptosis was extensively present in the CA1 region, the CA3 region and dorsocaudal brainstem were virtually unaffected. Additionally, significant reductions in NMDA receptor immunoreactivity were observed within the cortex and CA1 region following IH (Gozal et al., 2001a). This was consistent with the hypothesis that a chronic, slowly evolving glutamate excitotoxicity was one of the factors that underlie the structural and behavioral consequences of intermittent hypoxia in our model (Albin and Greenamyre, 1992).

Subsequent research from our laboratory suggested that the structural and neurobehavioral consequences of IH exposures in adult rodents involved a number of interrelated pathways, namely glutamate excitotoxicity, oxidative stress, mitochondrial dysfunction, upregulation of pro-inflammatory mediators, and altered regulation of pro- and anti-apoptotic gene cascades (Goldbart et al., 2003; Gozal et al., 2003a; Gozal et al., 2002; Gozal et al., 2001b). However, the explicit mechanistic involvement of ROS in the CNS end-organ injury has only been briefly explored. Row and colleagues showed that administration of a potent antioxidant markedly attenuated IH-induced spatial learning deficits in the rat (Row et al., 2003).

Additionally, aging rats exhibit unique susceptibility to IH-induced spatial deficits, an effect that is most likely explained by reduced antioxidant capabilities and downstream adverse effects on proteasome 20S/26S activities (Gozal et al., 2003b; Joseph et al., 1996).

Transgenic mice overexpressing CuZnSOD exposed to IH had a lower level of steady-state ROS production and reduced neuronal apoptosis in brain cortex compared with that of normal control mice exposed to IH (Xu et al., 2004). Furthermore, treatment with catechin

polyphenols or physical activity reduced lipid peroxidation in vulnerable brain regions even following IH (Burckhardt et al., 2008; Gozal et al., 2010), and conversely, a high oxidant stress diet was associated with increased CNS vulnerability to IH (Goldbart et al., 2006). Taken together, these findings suggest that the increased ROS production and oxidative stress propagation associated with IH contribute, at least partially, to IH-mediated neuronal apoptosis and neurocognitive dysfunction. In support of such assumptions, Veasey and collaborators confirmed some of these findings and further expanded on the critical role played by NADPH oxidase in the injury to locus coeruleus neurons and the excessive sleepiness that developed as a consequence of IH during sleep (Veasey et al., 2004; Zhan et al., 2005). Furthermore, treatment with the NADPH oxidase antagonist, apocynin, has recently been associated with neuroprotection in the context of IH in rats (Hui-guo et al., 2010). However, apocynin has been recently shown to have intrinsic anti-oxidant properties, and is probably not as specific as a NADPH oxidase inhibitor as originally assumed (Aldieri et al., 2008).

Although there is growing evidence that oxidative stress produces tissue injury in a wide variety of diseases, including sleep apnea (Bossy-Wetzel et al., 2004; Joseph et al., 1996; Lavie, 2003), the source of the oxidative stress in IH remains to be elucidated. As discussed above, one of the potential sources of oxidative stress in IH would be NADPH oxidase (Sies and de Groot, 1992). This pathway may be especially important in conditions in which there is overactivation of glutamate receptors and concomitant release of NO, such as cerebral hypoxia/ischemia and other excitotoxic processes (Bredt, 1999). While NO normally functions as a physiological neuronal mediator, as a free radical, NO is inherently reactive and can mediate cellular toxicity by damaging critical metabolic enzymes and by reacting with ROS, such as superoxide, to form an even more potent oxidant, peroxynitrite (Bredt, 1999). Activation of NADPH oxidase would then lead to the observed increases in carbonylation and lipid peroxidation injury observed after IH exposure and the downstream proinflammatory responses observed in the brain after exposure to long term IH (Zhan et al., 2005). Additional experiments are currently underway in our laboratories exploring the role of NADPH oxidase in cognitive deficits associated with not only IH, but also sleep fragmentation (Vijay et al., 2009).

5.3. Inflammation and Oxidative Stress in OSAS Patients

A growing body of evidence implicates inflammation and oxidative stress in the pathogenesis of OSAS in humans (Lavie et al., 2010; Schulz et al., 2000; Wali et al., 1998). For example, OSAS patients display increased circulating markers of oxidative stress and inflammation (Lavie and Lavie, 2009). This has led to the hypothesis that the repeated hypoxia/reoxygenation cycles encountered during sleep in OSAS patients alter the oxidative balance through induction of excess free radicals, in a similar fashion to that observed in ischemia/reperfusion injury (Lavie, 2003). Although the majority of work linking OSAS to oxidative stress has focused on the cardiovascular consequences of the disease, it is important to note that the brain is among the most sensitive organs to oxidative damage, primarily due to the large amount of polyunsaturated fatty acids, the high utilization of oxygen, and the relative paucity of antioxidant defense mechanisms (Butterfield et al., 2002). Given that oxidant stress has been implicated in the cognitive decline that occurs in both normal aging and neurodegenerative diseases (Beal, 1995), it is therefore likely that such mechanisms play a major role in the neurocognitive morbidities associated with OSAS. Consistent with this hypothesis, OSAS patients develop regional alterations in brain morphology (Macey et al., 2002; Macey et al., 2008; Rae et al., 2009).

5.4. Mitochondria and Intermittent Hypoxia

As previously discussed, mitochondria constitute one of the most important sources of ROS, and it would therefore be a reasonable assumption to explore their contribution to neuronal injury in the context of IH. To this effect, we employed cell culture and animal models to analyze the consequences of enhanced production of ROS on cortical neuronal cell damage and neurocognitive dysfunction. In primary cortical neurons, the transition phase from hypoxia to normoxia during IH appeared to generate more ROS than the transition phase from normoxia to hypoxia or hypoxia alone, all of which generated more ROS than control normoxic conditions. Using targeted inhibitors of the major pathways underlying ROS generation in the cell membrane, cytosol, and mitochondria, we showed that the mitochondria emerged as the predominant source of enhanced ROS generation during IH in mouse cortical neuronal cells, and that overexpression of MnSOD decreased IH-mediated cortical neuronal apoptosis, and reduced spatial learning deficits as assessed by the Morris water maze assay (Shan et al., 2007). In a very recent study, neonatal mice were exposed to intermittent hypoxia and intermittent hypercapnia for 10–14 days, and elicited marked increases in apoptotic cell death in the cerebral cortex. In addition, mitochondria were isolated from brain and showed significant reductions in both state 4 and state 3 respiratory activities along with increased superoxide production during nonphosphorylating state 4 in the absence of superoxide leakage (Douglas et al., 2010). We are unaware of any other studies specifically exploring the alterations in mitochondrial function and in ROS regulatory mechanisms in the context of IH. Thus, this area should provide ample and promising opportunities for not only identifying mechanisms of neuronal cell injury in sleep apnea, but also for formulation of therapeutic interventions aiming at abrogating or at least attenuating such serious morbid consequences of this frequent condition.

6. Summary

The cumulative evidence is supportive for ROS playing a major role in the deleterious effects of IH on selected CNS structures. The putative mechanisms underlying neuronal dysfunction and loss as well as reactive gliosis in animals and humans exposed to IH during sleep remain however poorly defined. Recent findings implicating NADPH oxidase and mitochondrial dysfunction as important sources of excessive ROS production in the context of IH provide a well needed impetus for accurate delineation of their roles and interactions, since such understanding may be instrumental to formulate novel therapeutic strategies in the future.

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Figure 1.

An overview of cellular sources of ROS. The electron transport chain (ETC) in the inner mitochondrial membrane (IMM) releases superoxide to both the matrix and the intermembrane space (IMS). NADPH oxidases (NOX) are localized in the cellular and endoplasmic reticulum membranes and release superoxide towards the luminal side of the membranes. Xanthine oxidase (XO) is localized on the outer surface of the cellular membrane, in the cytosol, and in peroxisomes and lysosomes. XO produces both superoxide and H_2O_2 . The cytosolic phospholipase A2 (cPLA2) is associated with the lipid layer of the cellular membrane and membranes of subcellular organelles. It releases superoxide to the cytosol. The secretory PLA2 (sPLA2) is localized in the extracellular space where it produces superoxide. Cytochrome P450 is localized in the cellular and endoplasmic reticular membranes and releases superoxide to the cytosol. Cyclooxygenase (COX) and lipoxygenase (LOX) are localized in the endoplasmic reticular membrane and release superoxide into the cytosol. Superoxide is reduced to H_2O_2 by MnSOD in the mitochondrial matrix, by CuZnSOD in the IMS and the cytosol, and by ecSOD in the extracellular space. H_2O_2 freely diffuses across membranes, which is depicted by dotted arrows. OMM: outer mitochondrial membrane.

Figure 2.

Mechanisms of superoxide production by the electron transport chain (ETC) in the inner mitochondrial membrane. Electrons (e^-) are donated by energy substrates to complex I and II, respectively, and are transported to complexes III and then IV, where they are accepted by oxygen, through a 4-electron reduction reaction, forming water. Premature leakage of electrons from the ETC may occur at complex I towards the matrix and at complex III towards both the intermembrane space and the matrix. Such premature electron leakage results in formation of superoxide ($O_2^{\bullet-}$) through one-electron reduction of oxygen. Solid red arrows: direction of electron transport; solid blue arrows: direction of reverse electron transport; dotted red arrows: direction of electron leakage; dotted blue arrows: direction of electron leakage through reverse electron transport; dotted black arrows: action of selected ETC inhibitors; thick dotted gray arrows: direction of proton translocation associated with electron transport.

Figure 3.

Mechanism of superoxide production by NOX2. The NOX2 enzyme is inactive under resting conditions, in which the cytosolic regulatory subunits are dissociated from the two membrane-bound subunits. Upon stimulation, the "organizer subunit" p47^{phox} moves toward the membrane and docks onto p22^{phox}, which leads to the recruitment of other regulatory subunits and the assembly of an active enzyme. The active NOX2 enzyme accepts electrons (e^-) donated by NADPH and, in a transmembrane redox reaction, transfers them to the outer side of the membrane, where the electrons are accepted by molecular oxygen, forming superoxide.

Figure 4. Representative tracing of obstructed events in a patient during NREM sleep (A). Examples of oxyhemoglobin trends during sleep in 3 different patients suffering from sleep apnea (B–D), illustrating the presence of IH and the unique patient-to-patient variability in oxygenation patterns associated with the condition.