The polymorphic and contradictory aspects of intermittent hypoxia

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Almendros I, Wang Y, Gozal D. The polymorphic and contradictory aspects of intermittent hypoxia. Am J Physiol Lung Cell Mol Physiol 307: L129–L140, 2014. First published May 16, 2014; doi:10.1152/ajplung.00089.2014.—Intermittent hypoxia (IH) has been extensively studied during the last decade, primarily as a surrogate model of sleep apnea. However, IH is a much more pervasive phenomenon in human disease, is viewed as a potential therapeutic approach, and has also been used in other disciplines, such as in competitive sports. In this context, adverse outcomes involving cardiovascular, cognitive, metabolic, and cancer problems have emerged in obstructive sleep apnea-based studies, whereas beneficial effects of IH have also been identified. Those a priori contradictory findings may not be as contradictory as initially thought. Indeed, the opposite outcomes triggered by IH can be explained by the specific characteristics of the large diversity of IH patterns applied in each study. The balance between benefits and injury appears to primarily depend on the ability of the organism to respond and activate adaptive mechanisms to IH. In this context, the adaptive or maladaptive responses can be generally predicted by the frequency, severity, and duration of IH. However, the presence of underlying conditions such as hypertension or obesity, as well as age, sex, or genotypic variance, may be important factors tilting the balance between an appropriate homeostatic response and decompensation. Here, the two possible facets of IH as derived from human and experimental animal settings will be reviewed.

intermittent hypoxia; sleep apnea; beneficial and pathological effects

Definition of Intermittent Hypoxia

SLEEP APNEA (SA) IS CHARACTERIZED by repetitive central apneic episodes or by repetitive occlusions of the upper airway (obstructive sleep apnea, OSA) that lead to intermittent hypoxia (IH), thus manifesting as recurrent blood oxygen desaturations. Although IH in patients with SA is typically characterized by short cycles of hypoxia and reoxygenation, the patterns of IH vary greatly across patients with SA. Thus, to enable comparisons across subjects or experimental conditions, IH needs to be defined by the duration of each cycle, the severity of hypoxemia in each cycle, the duration of IH per day, and the total number of days to years in which IH is present (Fig. 1). From these considerations, it becomes immediately apparent that IH patterns imposed during experimental protocols vary considerably between research groups and are primarily dictated by technical and biological reasons. For example, the rates of IH or cyclic hypoxia have ranged from minutes or hours to multiple events per hour throughout the circadian or ultradian cycle or during the sleeping period of rodents. The selection of those variables in translational studies has depended on the intent to mimic a specific human disease condition associated with IH (e.g., chronic obstructive pulmonary disease, OSA, asthma, hypoxia-reperfusion, obesity, cancer, etc.). Considering the high variability of IH paradigms employed in the experimental literature, we will here delineate each of those paradigms as we address several a priori contradictory consequences of IH, namely the beneficial and deleterious effects of IH.

Adaptation to IH: Beneficial Effects

Several studies have reported that IH-induced adaptations or interval hypoxic exercise training can provide some measurable protection in some disease states or enable improvements in selected sport-related performances. Globally, the protective effects of IH can be explained by the activation and propagation of homeostatic or adaptive responses elicited by the IH stimulus, usually through a process that has been generally termed preconditioning. Thus short exposures to mild IH paradigms can afford protection to specific cells, tissues, or organs against more severe hypoxia and ischemia. In addition, mild or training IH exposures have also been implicated in enhancements of both physical and mental capacities (81, 168). As indicated above, the IH patterns used in most of the following studies were characterized by relatively milder hypoxic exposures (>12% Fi02) and by single sessions per day or by brief exposures ranging from minutes to several hours. In the following sections, we will briefly provide illustrative examples of IH-induced beneficial effects.

Preconditioning effects. Animals subjected to various paradigms of acute IH become more resistant to injury in subsequent exposures to severe hypoxic/ischemic insults. For in-
Fig. 1. The protective and detrimental effects of intermittent hypoxia (IH) can be explained by the adaptation or maladaptation of the whole organism. Short- and low-frequency exposures to mild IH paradigms can afford protection to specific cells, tissues, or organs against more severe hypoxia. In addition, some homeostatic or adaptive responses elicited by IH have been described, such as release of stem cells. However, those mechanisms could be partially or totally abolished by other coexisting factors or diseases.

stance, compared with controls, mice treated with brief episodes of low-frequency IH (8% O₂ × 10 min/21% O₂ × 10 min, 6 cycles) survived substantially longer when exposed to lethal hypoxia. This phenomenon was accompanied by attenuated cellular and tissue injury in important organs, such as the lung and the brain (207). More detailed analyses revealed that IH-induced beneficial effects in the lung involved protection of the respiratory membrane integrity, especially hypoxia-sensitive type I epithelial cells, and preservation of gas-exchange function (207, 208). Furthermore, myocardium from mice treated with a similar IH paradigm (6% O₂ × 6 min/21% O₂ × 6 min, 5 cycles) or from rats treated with an IH paradigm with higher frequency but shorter duration (10% O₂ × 40 s/21% O₂ × 20 s, for 4 h) was shown to be protective from ischemia-induced infarction (17, 18, 23). Such IH-induced cardioprotection seemed to involve activation of pathways similar to those described in models of ischemic cardiac preconditioning (17, 18, 137) and required sufficient expression and activity of hypoxia-inducible factor 1α (HIF-1α) (23). Interestingly, IH paradigms of a longer period (7 days) but less severe FiO₂ (12% O₂) have been attempted in a recent study for postconditioning treatment against ischemic brain injury in rats (187).

Exercise training. IH has been extensively used for exercise training in an attempt to improve physical performance. Interval training in hypoxic conditions, a form of IH, promotes the induction of erythropoietin production and is associated with improved aerobic performance capacity in athletes (153). It has been suggested that this adaptation to IH exposures is mediated, at least in part, by increased peripheral chemosensitivity to hypoxia (82). In animals models, respiratory adaptations to IH have been studied in great detail (146) with purported increases in the acute hypoxic ventilatory response (82, 143, 146), ventilatory long-term facilitation (see below) (115), and improved maximal oxygen uptake at high altitude or sea level (52). Although all the physiological adaptations induced by short-term or chronic IH have been used to develop therapies with IH for exercise performance in athletes, there is still substantial controversy on the mechanisms underlying the putative IH-induced changes, the optimal IH schedules required for any specific goal, and the overall magnitude of changes elicited by IH (70). These discrepancies are likely due to the huge diversity of IH-exposure paradigms used and the large variety of exercise training schedules and performance targets studied. As such, no specific mechanistic inferences can be drawn from these empirical IH exposures.

Long-term facilitation of respiratory output and facilitation. As will be subsequently discussed, the central nervous system (CNS) undergoes degenerative changes in the context of long-term IH. However, attempts to minimize pathology by increasing the expression of growth/trophic factors that confer neuroprotection and neuroplasticity are also part of the response armamentarium of the CNS. For example, short-term IH (3 to 10 short cycles lasting a few minutes each and not more than 1–2 h/day) elicits respiratory motor plasticity, increasing the strength of respiratory contractions and breathing, a phenomenon that has been termed long-term facilitation (LTF). This type of short IH paradigm upregulates the expression and function of hypoxia-sensitive growth/trophic factors within respiratory motor neurons and is void of any detectable end-organ pathologies such as hippocampal cell death, neuroinflammation, or systemic hypertension. One of the most studied models of respiratory plasticity is LTF of hypoglossal and phrenic motor output following acute intermittent hypoxia (39, 55, 109, 117, 147). LTF is characterized by a progressive increase in respiratory motor output and is associated with increases in phrenic, hypoglossal, or carotid sinus nerve inspiratory-modulated discharge. This phenomenon has been described in several species, including humans, and can be influenced by other factors such as sex and age (199). Serotonin is currently the most characterized signaling mediator.
during IH-mediated LTF (103). However, LTF-driven increases in ventilatory output are mediated in part by slight increases in oxidative stress and other signaling pathways (106–108) and have been successfully harnessed as an approach to rehabilitate patients after spinal cord injury (69, 128, 129). However, LTF-generating paradigms could lead to long-term muscle fatigue with remodeling of the upper airway muscles and diaphragm and ultimately deleterious effects on motor control (53, 175–178). This dysfunction emerges as highly dependent on factors such as age (177) or sex (176).

Cardiovascular protection and improvement. IH can protect the heart against ischemia-reperfusion injury (197) by improving against ischemia-induced contractile dysfunction (31, 127), endothelial dysfunction (110, 111), arrhythmias (110, 116, 209), and cell death (45, 89). Also, IH treatment can promote higher resistance to arrhythmias during acute myocardial ischemia and prevent the development of atherosclerosis in rabbits (88). This protection has been ascribed to the higher myocardial blood flow, cardiomyoglogin, and expression of antioxidant proteins induced by IH (211). Other investigators have reported antihypertensive effects of conditioning IH exposures (9–10% O₂, 5–10 min, 5–8 times per day) in young spontaneous hypertensive rats, which have been associated with prevention of endothelial dysfunction and with increased accumulation of nitric oxide (NO) in vasculature (111). In addition, IH appears to provide a therapeutic effect on permanent coronary artery ligation-induced myocardial infarction by reducing the infarct size, myocardial fibrosis, and apoptosis (202). Due to the benefits reported by IH on myocardial infarction and its simple intervention with fewer adverse effects, IH paradigms have been proposed as treatment for patients suffering from these conditions.

The early phase of reperfusion after myocardial ischemia can generate a large amount of reactive oxygen species (ROS), which can contribute to increase in the myocardial injury (14, 189, 212). However, at the same time, ROS also can trigger cardioprotection induced by ischemic or pharmacological conditioning (34, 68, 188). The possible role of ROS has been explained as depending on concentration, such that ROS emerge as cardioprotective at low levels but detrimental at high levels (36, 165). In support of such assumptions, Naghshin and colleagues (118, 119) reported that chronic IH treatment is sufficient to improve cardiac function in healthy mice and transgenic mice with underlying heart failure.

Neurovascular protection and cognitive improvement. The vascular protective role of IH is not only confined to the heart. IH-mediated adaptations can also lead to improved cerebral flow responses to ischemia or stroke. Previous studies have reported that preconditioning with long hypoxic periods (hours) can minimize cortical infarction (102) as well as dampen learning and memory impairments related to severe CNS ischemia in rats (163, 164). These benefits have been related to an increased proliferation of neural stem cells in the subventricular zone and dentate gyrus observed after rats were subjected to hypoxia 4 h per day for 2 wk (210) and have also been related to the upregulation of c-Fos expression (163). However, as discussed below, higher frequency or duration of IH can induce oxidative stress and neuronal apoptosis promoting detrimental effects on memory (201).

Low-frequency IH in rats after brain ischemia can decrease infarct volume (186), which in turn can attenuate ischemia-induced spatial learning and memory deficits in those animals (187). As occurs with IH preconditioning, IH interventions following stroke occurrence induced hippocampal neurogenesis that ameliorated memory losses (187). Furthermore, application of posts ischemia IH can upregulate hippocampal brain-derived neurotrophic factor, suggesting that IH induces synaptogenesis (187). As a corollary to these findings, in a recent study carried out in patients with OSA, Hoth et al. (72) found that more severe hypoxemic events may result in memory improvements.

Newborns can be subjected to transient oxygen fluctuations and deprivation during birth (acute IH). Interestingly, noninjurious severe short-lasting neonatal hypoxia conferred resistance to brain senescence in aged male rats. Indeed, Martin et al. (113) reported in rats at 21 days of age increased cortical thickness and cell densities with strong synapsin activation in several brain subregions that were potentially associated with improved preservation of cognitive functioning.

Other potential benefits of IH. Application of brief IH (cycles of 5 min, hypoxia 10%) in humans has been tested as immunotherapy. IH appears to enhance innate immunity by mobilizing hematopoietic progenitors, which activate immune cells such as neutrophils and increase circulating immunoglobulins (172). The authors suggested that IH can increase immune defenses without exacerbating inflammation. However, the establishment of IH treatment in patients is potentially fraught with substantial difficulties because the application of IH in humans needs to be exhaustively controlled and monitored. Another study carried out in humans showed similar results, whereby IH promoted the release of progenitor cells (191), such that short-term IH with muscle electrostimulation increased the number of hematopoietic CD34+ stem cells in circulation and could help increase hematopoietic stem cell homing in injured tissues (191). We should remark that short-lasting IH exposures mimicking SA were associated with recruitment of bone-marrow derived pluripotent stem cells that exhibited upregulation of stem cell differentiation pathways, particularly involving CNS development and angiogenesis (58, 59).

Maladaptation to IH: Pathological Effects

There is increasing evidence that IH plays a mechanistic role in the development of cardiovascular, metabolic, and cognitive consequences in the context of the OSA through activation of oxidative stress and inflammatory pathways (Fig. 2). The IH pattern in most of the animal-based studies aiming to replicate OSA included higher cycle frequency (from seconds to few minutes), lower FIO₂ being applied, and restriction of IH to the sleep or rest period (149). As mentioned, the selected IH parameters aim to recapitulate some characteristics of OSA, as defined by the apnea hypopnea and oxygen desaturation indices usually seen in the clinical practice settings (161).

Link between IH and inflammation. Recurrent blood oxygen desaturation is the prototypic feature of IH. The changes of oxygen supply in circulation can elicit swings in oxygen partial pressure in tissues and more markedly in those with higher metabolic rate and perfusion (i.e., brain, liver, kidney) (5, 9). Although some homeostatic responses to IH (and hypercapnia)
Thus HIF-1, a commonly studied transcriptional activator, is involved in angiogenesis and homeostatic responses. HIF-1 is a master oscillator linking IH to ROS production, activation of inflammation, and oxygen oscillations that may develop over time, the oscillating changes in tissue oxygen availability are widely considered as an important source of ROS. These molecules can be generated from different subcellular compartments and organelles, such as mitochondria, cellular membrane, lysosomes, peroxisomes, and the endoplasmic reticulum (11, 15, 51, 91, 167).

The HIF family can account, at least in part, for some of the processes linking IH to ROS production, activation of inflammation, and downstream upregulation of other molecules involved in angiogenesis and homeostatic responses. HIF-1 is a commonly studied transcriptional activator, which comprises an O2-regulated α-subunit and a constitutive β-subunit (193). Thus HIF-1α can be induced by hypoxia as a consequence of decreased O2-dependent degradation of HIF-1α (35, 204). HIF-2α is another well-studied member of the HIF family induced by continuous hypoxia, which also can interact with HIF-1α (182). Although continuous hypoxia promotes the increased expression and activity of both molecules (71), acute IH upregulates HIF-1α and downregulates HIF-2α protein via calpains (125). Furthermore, contrary to sustained hypoxia, long-term IH during sleep has been reported to induce early activation of HIF-1α in the brain that is then followed by reductions in HIF-1α expression or its target genes over time (41, 99, 122). If we consider that HIF-2α regulates the transcription of several antioxidant enzymes including SOD-2 (169), the downregulation of HIF-2α in the context of acute IH may also contribute to increases in ROS via insufficient transcription of antioxidative enzymes. Therefore, the differential regulation of both transcriptional factors in acute IH and the potential biphasic or multiphasic temporal trajectories of HIF in chronic IH may account for the higher levels of ROS in IH compared with sustained hypoxia. Peng et al. (141) showed that IH enhances carotid body responses to hypoxia and elevates local ROS production that is mediated by HIF-1α accumulation, and such responses appear to underlie some of the cardiorespiratory responses to IH.

In addition, most cells will respond to increased ROS by upregulating the redox-sensitive transcriptional factor NF-κB, a pivotal molecule in the proinflammatory response. In the context of hypoxia, NF-κB can be also modulated by HIF-1α (60). In the nucleus, NF-κB upregulates the transcription of several proinflammatory genes responsible for encoding inflammatory cytokines (tumor necrosis factor-α, interleukin-6, and interleukin-8), chemokines, surface adhesion molecules, and other enzymes such cyclooxygenase-2 (COX-2). Interestingly, the redox-sensitive NF-κB can activate endothelial cells, leukocytes, and platelets expressing adhesion molecules and proinflammatory cytokines and contribute to the endothelial dysfunction and other cardiovascular morbidities that have been ascribed to long-term IH (21).

Metabolic consequences. An increased risk for developing metabolic syndrome has been widely associated with the presence of OSA in obese adults and children (20, 37, 50, 62, 126, 139, 162, 183, 185). For example, when comparing obese people with and without OSA, Vgontzas et al. (190) showed that the major difference between both groups was the higher amount of visceral fat depots in patients with OSA. This increase in visceral fat content was strongly correlated with the degree of respiratory disturbance or severity of nocturnal intermittent hypoxemia (190). However, the potential mechanisms implicated in the pathogenesis of metabolic syndrome in the context of OSA and IH remain incompletely defined in part due to the high prevalence of obesity in patients with OSA. Therefore, the use of animal models has been key to better understand the role of IH for each of the metabolic alterations encompassed in the context of metabolic syndrome. In a mouse model of OSA, IH exposures induced dyslipidemia by upregulating lipid biosynthesis in the liver through HIF activation,
increasing lipolysis within the adipose tissue, and inhibiting lipoprotein clearance (47). In addition, the detrimental effects of IH on insulin sensitivity and glucose homeostasis have been illustrated, particularly in the concurrent presence of obesity (48, 73). Other studies carried out in cultured cells and reproduced in animals showed that IH-related intermediate mechanisms can alter insulin signaling in adipocytes (151). Also, IH stimulates pancreatic β-cell replication (203) via upregulation of Reg family genes and the hepatocyte growth factor gene (135). Similarly, coinciding with the increase in proliferation, the subcellular localization of the cell cycle regulator cyclin D2 was increased in the nucleus of pancreatic β-cells (200). In addition, pancreatic β-cell death was increased approximately fourfold. However, manganese superoxide dismutase transgenic overexpression did not alter the effects of IH on β-cell proliferation but completely abrogated the IH effects on cell death. Reinke et al. (70) reported that IH resulted in lower levels of tissue oxygenation, promoting insulin resistance in both lean and obese mice. In lean mice, IH increased serum leptin levels, oxidative stress markers, and adipose tissue inflammation (152). Similarly, Polak et al. (144, 194) found that 14 days of IH induced insulin resistance, impaired β-cell function, enhanced hepatocyte glucose output, and increased oxidative stress in the pancreas. However, those reported changes were not completely reversed by cessation of IH (144, 194). An additional aspect of IH is that it is associated with gestation, where it has been found to increase the risk of the offspring to metabolic diseases developing during adulthood (74). Cumulatively, the data suggest that IH can promote some components of the metabolic syndrome, playing an independent cofactor role in conjunction with obesity. However, we need to again emphasize that IH-reported effects could depend on the duration, severity, and frequency of IH. For instance, different frequencies of IH resulted in divergent patterns of metabolic dysfunction (29, 152).

Cardiovascular consequences. Patients with OSA exhibit significantly higher risk of hypertension, arrhythmia, and myocardial infarction (131, 173). From murine models, it is well known that long-term IH can promote increased blood pressure, biventricular hypertrophy, left ventricle contractile dysfunction (30, 32, 54, 56, 198), and infarction (77) mainly through the previously mentioned ROS-mediated mechanisms and/or peripheral chemoreceptors (57). IH sympathetic-induced activation, alterations in carotid body, reduced NO bioavailability, and/or increased myogenic tone have been proposed as potential mechanisms promoting hypertension (75, 97, 134, 148). Similar to IH and metabolic disorders (145), short-term exposures to IH in rats (1 and 4 days) seem to be protective (95). However, longer periods (>1 wk of IH exposures) resulted in detrimental effects. The outcomes observed by different authors are highly varied and likely depend on the particular IH parameters employed, such as duration, frequency, and severity of hypoxia. With consideration of these factors, long-term IH exposures in murine models showed increased right ventricular systolic pressure, right ventricular mass, neovascularization of distal pulmonary vessels (54), cardiomyocyte diameter (133), systemic and pulmonary vascular pressures (24, 132), interstitial space (33), cardiac hypertrophy, cardiac and perivascular fibrosis (133), inflammation and dysfunction (198), and a decrease in endothelial nitric oxide synthase (eNOS) expression in ventricular and aortic tissues (133, 192).

Those adverse cardiovascular effects are not limited only to the heart. In addition to hypertension, IH can promote vascular dysfunction, which has been associated with a higher risk of atherosclerosis (49) and stroke in patients with OSA (40). The development of endothelial dysfunction has been ascribed to a variety of mechanisms including oxidative stress, inflammation, growth factors, adhesion molecules, as well as some of the pathways described above. A possible relationship between endothelial dysfunction and IH involves excessive formation and propagation of ROS species (46, 93). In fact, patients with OSA present with attenuated expression of eNOS and an increased expression of nitrotyrosine (an oxidative stress marker) in endothelial cells (76). Interestingly, treatment with continuous positive airway pressure (CPAP) for 4 wk was sufficient to improve endothelial function and reduce oxidative stress (76). Similarly, Del Ben et al. (43) showed higher levels of urinary 8-iso-PGF-2α and serum-soluble NADPH oxidase 2-derived peptide with lower NO metabolites nitrite and nitrate (NOx) in patients with severe OSA (43). They also found a negative association between flow-mediated brachial artery dilation and OSA severity. After 6 mo of CPAP treatment, oxidative stress and arterial dysfunction were partially reversed (43). In rats, inhibition of xanthine oxidase, a major source of superoxide in endothelium, is able to attenuate the endothelial dysfunction caused by IH (46).

Arterioles and pulmonary arteries from rats exposed to IH were more sensitive to endothelin-1 (ET-1) suggesting an impaired endothelium-dependent vasodilation and increased vasoconstrictor responsiveness (2, 181, 196). Allahdadi et al. (1, 3) suggested that hypertension derived from exposure to IH seems to cause alterations in ET-1 signaling, increasing calcium sensitivity during ET-A activation. This phenomenon, which is similar to that experienced in patients with SA (79, 83, 90), appears to involve PKC-β that mediates the augmented vasoconstrictor reactivity to ET-1 in the pulmonary circulation of IH rats (180). This is in contrast to the role of PKC-δ in systemic arteries (2). Capone et al. (27) showed that mice exposed to chronic IH exhibit altered key regulatory mechanisms of the cerebral circulation through ET-1 and NADPH oxidase-derived radicals. Also, a recent study by Marcus and colleagues (112) proposed that endothelial dysfunction associated with IH can be also dependent, at least in part, on renin-angiotensin system signaling (112). Dematteis et al. (44) found early functional cardiovascular remodeling in mice in response to IH presenting as delayed changes in peripheral vasoreactivity. However, Julien and collaborators (78) revealed that vasoconstriction is enhanced in mice exposed to IH but that no dysfunction of endothelium-relaxation occurred. In addition, IH can promote vessel inflammation, enhancing the progression of atherosclerosis, which has been suggested to be mediated by upregulation of inflammatory adhesion molecules (13, 92, 94). Cell type regulated on activation normal T cell expressed and secreted (RANTES)/CCL5 has been proposed as a determinant cytokine for IH-induced preatherosclerotic remodeling (12). In cerebral cortex, IH-induced ROS can produce important alterations of cerebrovascular regulation via endothelium-dependent vasodilation and neurovascular coupling, which seems mediated in part by increased levels of ET-1 (27). Phillips et al. (142) showed that chronic IH can
impart endothelium-dependent dilation in cerebral and skeletal muscle resistance arteries in rats. Although OSA has been associated with endothelial dysfunction independently of other confounding factors such as obesity (124), it is well known that some metabolites released in the context of IH could also indirectly participate in this process. Also, experimental data obtained from in vitro and in vivo skin biopsies of patients with OSA showed a differential gene expression profile according to severity of hypoxemia and related to OSA-induced vascular dysfunction (80).

Recently, substantial interest has emerged in relation to the possibility that IH may harness the recruitment of progenitor and stem cell populations. We have previously shown that IH triggers the mobilization of some bone marrow-derived stem populations including very small embryonic-like pluripotent stem cells in mice (58). Similarly, in humans, bone-marrow derived hematopoietic stem cells are activated and appear in the circulation (172). In addition, acute exposures to recurrent apneas in rats promote the mobilization of bone-marrow mesenchymal stem cells (28). However, the implications of the increased number of progenitor cells are unclear. In patients with OSA, changes in endothelial progenitor cells (EPCs) (104) have emerged and been correlated to endothelial dysfunction (86). Current assumptions posit that EPCs could be released during acute IH exposures and facilitate repair mechanisms involved in restoration of endothelial injury but could also act as modulators of the inflammatory response. EPCs from healthy individuals were increased after exposures to intermittent hypoxia in vitro (19). However, the increase in EPCs in patients with OSA has been somewhat inconsistently reported. It is probable that the prolonged exposure to IH, either alone or in coexistence with underlying comorbidities, may have affected the global densities of stem cell populations in the circulation. Thus the high complexity of OSA in the context of time, severity, and coexistence of other diseases may account for the incongruence of findings and the controversy about the role of EPCs in the circulation of these patients (4, 19).

Cognitive consequences. Increased awareness to the potential neurocognitive consequences of episodic hypoxia led to development of animal models aiming at replicating the cardiorespiratory and sleep patterns of patients with OSA, such as to enable investigation of the more specific roles played by each of these abnormalities on the CNS. Indeed, SA has been associated with a broad range of neurocognitive difficulties, including excessive daytime sleepiness, personality and psychosocial maladjustment patterns, and mental impairments in both adults and children (16, 84, 87), which have been corroborated by imaging evidence of structural and functional aberrations (38, 61, 105). Regional reductions in gray matter that improved after CPAP treatment along with parallel amelioration in memory, attention, and executive functioning have also been reported (26).

Gozal and colleagues (63) reasoned that IH exposures would elicit the oxyhemoglobin desaturation patterns that closely resemble the patients with moderate to severe OSA, such that inferences could then be made on the anatomical and functional pathologies that such exposures would elicit. With the use of this IH paradigm in rats, exposures for 14 days to an IH profile consisting of alternating 90-s epochs of hypoxia (10% O2) and room air during the habitual sleep times induced substantial impairments on a hippocampal-dependent learning task, the spatial reference version of the Morris water maze (63). In contrast, no alterations emerged in IH-exposed animals on a simple cued version water maze task, indicating that these impairments could not be ascribed to alterations in sensorimotor function. Since these initial findings, multiple studies have confirmed that both rats and mice display cognitive deficits consistent with impaired functioning of the hippocampus and/or prefrontal cortex following exposures to IH (42, 157, 159). This suggests a reduced probability for long-term potentiation of field-evoked potentials following a titanic pulse train stimulation of Shaffer collaterals in the CA1 region of the hippocampus (138), where alterations in G protein-mediated signal transduction contribute to IH-related hippocampal dysfunction (67).

In developing rats, exposures to IH from postnatal day 10 to 25, a developmental period that corresponds to the peak prevalence of OSA in children, was not only associated with prominent neurobehavioral deficits in the spatial reference version of the Morris water maze (159) but also coincided with unique susceptibility to IH as reported by neuronal apoptosis cell counts (65). Furthermore, another period of increased vulnerability to IH occurs during aging, whereby aging rats exposed to room air or IH displayed significant spatial learning impairments compared with similarly exposed young rats, but decrements in performance associated with IH conditions were markedly greater in the aging rats (64). IH during sleep induced decreases in proteasomal activity that were particularly pronounced in the aging animals, indicating the formation of protein aggregates and attendant neuronal cell dysfunction and increased apoptosis.

Increased expression of oxidative stress markers occurs in the brains of rodents exposed to IH (150, 160, 174, 201) and has been mechanistically implicated in the cognitive and behavioral deficits described heretofore (120, 195). In addition, increased inflammation as evidenced by increased prostaglandin E2 neural tissue concentrations, a marker for the expression and activity of the inflammatory protein COX-2, is evident in hippocampal and cortical regions following exposures to IH (100) and also accompanied by lipid peroxidation of polyunsaturated fatty acids. In addition, increased carbonylation and nitrosylation-induced oxidative injury emerges in IH-susceptible brain regions and promotes increased somnolence (166, 205). Although the exact cellular sources of such IH-induced detrimental effects are still incompletely defined, it is likely that a portion of such deleterious effects is ascribable to activation of astroglia and subsequent loss of buffering functions that ultimately contribute to pathological processes, such as increased glial proliferation and microglial activation (63, 179). Astroglial and microglial cells play critical roles in regional blood flow regulation and inflammatory processes in the brain as well as critical coordination of bioenergetics through lactate transport (22, 140, 155). Activated microglia express high levels of the inducible isoform of NOS (iNOS) and COX-2, with both enzymes initiating pathways ultimately leading to generation and propagation of ROS. Furthermore, cytokine release magnifies the production of ROS superoxide and hydrogen peroxide as well as the activity of iNOS and COX-2 by microglia, thus perpetuating inflammation and aggravating ongoing oxidative stress (66). Consistent with these findings, experiments in a murine model of IH demonstrated...
that both pharmacological and genetic inhibition of iNOS afforded protection against IH-induced learning deficits (101), and pharmacological inhibition of COX-2 markedly attenuated IH-induced spatial learning deficits in rats (158).

There is now compelling support for the role played by the pro- and antioxidant cellular systems in neuronal injury associated with IH. In addition to the aforementioned considerations, we should note that transgenic mice overexpressing Cu,Zn-superoxide dismutase that were exposed to chronic IH had a lower level of steady-state ROS production and reduced neuronal apoptosis compared with wild-type mice (174). Furthermore, NADPH oxidase-dependent production of superoxide radical (O$_2^-$) has been identified as a major contributor to oxidative injury in the brain and other target organs under conditions of inflammation and severe hypoxia. Long-term IH increases the expression of NADPH oxidase (i.e., its p47phox subunit), the major enzyme underlying oxygen radical production, suggesting that activation of NADPH oxidase may, at least in part, underlie the increased neuronal inflammation and oxidative stress observed in animal models of OSA (120, 121, 123, 206).

Alterations in neurotransmitter systems may also play a role in the neurobehavioral disturbances seen after IH exposures and have implicated disruption of norepinephrine and dopaminergic pathways in the development of hyperactivity and working memory dysfunction (42, 85, 98, 159). The available evidence suggests that dysregulation of dopaminergic function is of significance in the context of IH-induced neurobehavioral deficits (156).

Cancer. IH is becoming a focus of great interest in cancer research because most solid tumor types develop intratumoral IH in the context of episodic changes in vascular supply to rapidly proliferating tumor regions (114, 154, 184). In two recent seminal epidemiological studies, OSA has been associated with an increased incidence (25) and enhanced mortality (130) in cancer. In this context, the cyclical hypoxia that characterizes OSA has been proposed as the major correlate of processes involving tumor invasion and metastasis (96, 154). Previous studies in mice showed that IH exposures lead to accelerated melanoma tumor growth (6) and metastatic potential (8), thereby lending biological plausibility to the epidemiological studies and further supporting the putative role of episodic hypoxia in tumor biology (136, 170, 171). Specifically, IH was able to accelerate the tumor growth in a melanoma model and also increased the metastatic potential from two different experimental metastatic models (intravenous and subcutaneous tumor injection). Although the preliminary data suggest that hypoxia-inducible genes could participate in the increased levels of VEGF and therefore in tumor vascularization (7), the potential mechanisms involved in IH-induced changes in tumor growth remain to be explored in further studies. Very recent work from our laboratory further suggests that IH-induced alterations in innate immunity, more specifically in tumor-associated macrophage polarity may account for some of the changes in tumor biology (10).

Summary

The current evidence supports the notion that IH elicits divergent responses that are stringently dependent on the contextual setting in which IH occurs. The characteristics of IH exposures, particularly focused around severity of hypoxia, duration, and cycle frequency, emerge as the fundamental determinants of IH-related outcomes. As such, short, mild, and lower cycle frequency would be generally anticipated to generate beneficial and adaptive responses, whereas chronic, moderate to severe, and high frequency IH will induce maladaptive disruption of homeostatic mechanisms, leading to end-organ dysfunction (Fig. 2). Efforts are clearly needed to refine and delineate the specific characteristics of IH that account for the wide spectrum and divergence of responses to harness the potential benefits of IH while developing therapeutic targets against its deleterious consequences.

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Author contributions: I.A. prepared figures; I.A. and Y.W. drafted manuscript; I.A., Y.W., and D.G. approved final version of manuscript; D.G. edited and revised manuscript.

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INTERMITTENT HYPOXIA


Review

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