Can O₂ Dysregulation Induce Premature Aging?

Chronic intermittent or episodic hypoxia, as occurs during a number of disease states, can have devastating effects, and prolonged exposure to this hypoxia can result in cell injury or cell death. Indeed, intermittent hypoxia activates a number of signaling pathways that are involved in oxygen sensing, oxidative stress, metabolism, catecholamine biosynthesis, and immune responsiveness. The cumulative effect of these processes over time can undermine cell integrity and lead to a decline in function. Furthermore, the ability to respond adequately to various stressors is hampered, and this is traditionally defined as premature aging or senescence. This review highlights recent advances in our understanding of the cellular and molecular mechanisms that are involved in the response to intermittent hypoxia and the potential interplay among various pathways that may accelerate the aging process.

About 10-15 years ago, we often wondered whether the intermittent hypoxia/hypcapnia that represented one of the hallmarks of obstructive sleep apnea/hypventilation (OSA/H) should be treated, especially since it was intermittent and most patients were relatively normal in terms of blood gases during the day. Often these patients did not even show HCO₃⁻ accumulation or an increase in hematocrit, and their erythropoietin levels were within the normal range. Despite the fact that intermittent hypoxia seems to exert much less of an impact on the physical growth of animals and humans than constant hypoxia (58), there is accumulating evidence now that intermittent hypoxia can adversely affect the function and development of the organism (47, 77, 136, 139). As will be described below, chronic intermittent hypoxia can lead to oxidative stress and inflammation that could predispose the organism to cumulative injury and acceleration of the aging process. Aging or organismal senescence has been defined as the process whereby pathological changes in the structural integrity of cells and the organism as a whole lead to a decline in function and reparative capacity. This process depends on many factors, but it is generally thought that aging is caused by the accumulation of macromolecular damage, especially to mitochondrial DNA, cellular proteins, and organelles (92). This cumulative damage and dysfunction eventually depletes the cell's and organism's ability to contend with stress. This results in impaired homeostasis, a decline in function and structural integrity, an increased risk of disease, and subsequent death.

Recent evidence in both clinical and experimental studies indicates that intermittent or cyclical hypoxia also leads to a variety of processes that lead to increased oxidant injury, an increase in various chemokines and cytokines, increased insulin resistance, vascular disease, and accelerated aging. We therefore hypothesize that inappropriate oxygenation or O₂ dysregulation, whether it is hypoxia or hyperoxia, will serve to accelerate the process of aging.

Although there are many factors that determine aging, whether in the developing or mature individual, some of the important factors relate to inflammatory mediators, lipid and glucose metabolism, and to the “age” of the vasculature that depends on such particular alterations. A prime example, clearly, is that of diabetes and the effects of glucose metabolism on the “age” of the vasculature. In the past few years, there have been a number of investigations that demonstrate that intermittent hypoxia, as a partial surrogate for airway obstructive disease during sleep, can lead to major vascular and inflammatory changes in various tissues. In our mind, this raises the question as to whether OSA/H and similar diseases that are characterized by an intermittent hypoxic stress lead to premature or accelerated aging. Indeed, there is an increased risk in patients with OSA/H for hypertension, stroke, myocardial infarction, arrhythmias, neurocognitive morbidity, and sudden death (17, 117, 136, 141, 164, 167). It is obvious then that OSA/H is much more than just intermittent hypoxia, but even if it is exemplified by this stress only, there is an important need to investigate fully and comprehensively the impact of sleep-related breathing disorders on cardiovascular and central nervous systems.

In this review, we detail some of the recent and important findings related to the effect of intermittent hypoxia as a stress on the organism but, in particular, on both the central nervous system and the cardiovascular system.
system. We will also put these findings in the context of a unifying hypothesis that underlies the pathobiology of intermittent hypoxia.

**Pathobiology of Intermittent Hypoxia: A Unifying Hypothesis**

There are many alterations that result from a stress such as hypoxia. In contrast to constant hypoxia, which depends on the duration of the stress, it has been shown that intermittent hypoxia (IH) seems to increase insulin resistance during the exposure (98, 159). Interestingly, a rebound adaptation occurs when this hypoxia stops and regular oxygenation sets in: an increase in insulin sensitivity ensues (155). Of note, this rebound does not happen in the ob/ob mouse, indicating that this depends on an intact leptin pathway for it to happen.

IH can increase insulin resistance via different mechanisms (modeled in **FIGURE 1**). For example, IH increases serum and liver fatty acid levels (155). This increase in fatty acids is brought about by an increase in sterol regulatory element binding protein-1 (SREBP-1), a transcription factor of lipid synthesis, and a downstream enzyme, stearoyl coenzyme A desaturase (SCD-1). It has been suggested that endothelin-1 (99, 183) is also an important factor in insulin resistance during IH. Although the increase in

**FIGURE 1.** Modular description of the interactions among signaling pathways that participate in the response to intermittent hypoxia.

- NF-κB/IκBα
- ROS
- Mitochondria
- NOX2
- HIF-1α
- Sympathetic activation
- Endothelin-1
- SREBP-1
- Glycolytic enzymes: Glut-1, Leptin
- Serum catecholamines
- SCD-1
- Serum and liver fatty acids
- Lipolysis
- Cortisol
- Triglycerides
- Glucose release
- Glucose uptake
- Increased insulin resistance
- Vessel disease/ Atherosclerosis
- Cell injury/Death/ Remodeling
- Aging process
- Blood pressure
- IL-6; TNFα; IL-1β
- NMDA
- Calcium
- Lipid peroxidation
- Endothelial cells
- Leukocytes
- Platelets
- IL-8; Selectins I/V-CAM
SREBP, SCD, and endothelin are a result of HIF-1 activation, which can certainly take place in IH, HIF-1 can also activate a number of mechanisms that would enhance insulin sensitivity (see FIGURE 1, pathway 1). For example, HIF-1 is well known to enhance glycolytic enzymes, glucose transport (Glut-1), and leptin (6, 176). From the point of view of mechanisms, it may be difficult to predict how HIF-1 activation will affect insulin resistance, although IH does result in increasing insulin resistance. Hence, either these changes resulting from HIF-1 activation lead to this resistance or else other factors in addition to those resulting from HIF-1 activation tilt the balance toward this phenotype. Of importance, other mechanisms in IH are also crucial in determining glucose and lipid metabolism.

One of the important discoveries about the consequences of IH is that it is as much due to low O₂ as to the production of reactive O₂ species (ROS). Whether the increase in serum catecholamine (see FIGURE 1, pathway 2) (119) ROS will produce lipid peroxidation (29, 167, 198, 210) in, for example, liver, brain, and heart that sets the stage for tissue injury (29, 167, 171). Furthermore, it is likely that ROS activates NF-κB, a major transcription factor that, when activated, translocates to the nucleus to regulate a number of inflammatory genes (see FIGURE 1, pathway 3) (107), including IL-6, TNF-α, and IL-1β. NF-κB can promote, in addition, the activation of endothelial cells, leukocytes, and platelets (208). It is also interesting that obesity, which is known to induce the stimulation of these inflammatory mediators, may add insult to injury when OSA/H is also present since IH, irrespective of body habitus and weight, increases these inflammatory proteins in the serum of patients (168) because of hypoxia and ROS. TNF-α can then set a positive feedback loop since it stimulates a kinase (IKK) that phosphorylates IκBα to release NF-κB to the nucleus for gene activation (85). NF-κB also stimulates other cytokines and adhesion molecules such as IL-8, selectins, and I/V-CAM, which have been associated with vessel disease, atherosclerotic plaques, and OSA/H (35, 52).

The importance of these changes in NF-κB, cytokines, and enzymes (IKK), which occur in obesity as well as in IH without obesity, is that not only do they induce and perpetuate the process of atherosclerosis but they can also induce insulin resistance by increasing lipolysis (see FIGURE 1, pathway 4) (122). This increase in serum lipids, as well as increased blood glucose and insulin resistance, enhances vascular inflammation and injury, starting a vicious cycle that promotes premature aging.

OSA/H and IH can enhance the aging process by increasing systemic blood pressure, which by itself will generate vascular and renal changes (e.g., renin and angiotensin II) that exacerbate vessel disease (181, 184). This systemic increase in blood pressure results from sympathetic activation and from an increase in serum catecholamine (see FIGURE 1, pathway 5) (113, 137, 151). Catecholamines also have other effects on blood glucose and insulin release via other hormones. For example, catecholamines increase the release of glucose and decrease its uptake. They also increase the release of triglycerides and through an increase in cortisol can increase lipolysis, which in turn will decrease insulin sensitivity (153).

From this summary, it is not surprising therefore that IH and OSA/H can be injurious and can start a cascade of events that if perpetuated leads to vessel disease and premature aging. This is brought about by altering glucose homeostasis, dyslipidemia, induction of a variety of inflammatory mediators and adhesion molecules that are essentially atherogenic. Furthermore, one of the important observations that has been recently made is that innate immune receptors, which are crucial for such inflammation to occur and take effect, have previously been assumed to be only found on leukocytes or immune cells. It is clear now that such receptors are also present in specific organs and cells. Toll-like receptors are not only present on immune cells but also, for example, on neurons and glia in the CNS. Hence, IL-1β, for instance, can be stimulated by the activation of TLR-2 on neurons, and this has major implications on neuronal and glial function under stressful conditions such as in hypoxia or ischemia (207). Therefore, inflammatory responses may not only be happening in the vasculature but also in specific organ tissues (as in the CNS in the latter example) and may initiate a number of reactions that lead to cell injury and death in the tissue itself. It is still unclear though how these immune receptors are activated; i.e., these receptors that have been generally assumed to be activated only by microbes or by bacterial wall antigens. It is possible that the activation of such immune receptors is based on some other molecules that are matrix-based (44) or is a result of cell injury and substances that are shed by dying cells in the interstitial space. If IH during childhood can evoke similar changes in glucose and lipid homeostasis and thereby predispose to premature aging, it is conceivable that studies in neonates or in children may bring to light biomarkers of potential injury.

**Cellular and Molecular Mechanisms Underlying the Pathobiology of Intermittent Hypoxia**

**Definition and models of IH**

IH is generally defined as repeated periods of hypoxia with alternating periods of normoxia. Several approaches have been utilized in the recent past to emulate the episodic hypoxia that occurs during...
diseases such as obstructive sleep apnea/hypoventilation (OSA/H) and the apnea of prematurity. Therefore, protocols have tended to vary from very short periods of hypoxia/normoxia (2-10 min) to very long periods that last for hours at various levels of \( \text{O}_2 \) concentration (139). As will be described in the text, our laboratory has utilized a model whereby alternating brief exposures of 4 min of hypoxia or hypercapnia with 4 min of normoxia and normocapnia (6 cycles/hour) that is maintained either for 12 h/day during the light phase, because mice are nocturnally active, or continuously for 24 h/day for up to 4 wk. We believe that this paradigm is close to what is seen in OSA/H patients and is technically feasible. Other investigators have utilized a variety of paradigms tailored to fit their experimental needs. Such IH models contrast with those of a constant hypoxic stress, which resembles the stress for example at high altitude and which is characterized by a rather constant level of blood \( \text{O}_2 \). Although the severity of the constant stress depends on the level of \( \text{O}_2 \), this constant stress does not, by definition, oscillate and does not induce the same variety of alterations as with a frequently intermittent hypoxic stress. Acute studies of IH in animals have varied from very short exposures (1 h) up to several weeks (4, 29). Mild IH exposures have been used in human studies such that the \( \text{O}_2 \) saturation (\( \text{Sa}_\text{O}_2 \)) is usually no less than 80%, and the duration of exposure was varied from 20 min to 1 h (190, 203). In chronic IH studies in animals, \( \text{O}_2 \) levels have varied from 10% to 2–3%, and durations have ranged from 6 days up to 84 days for either 7, 8, 12, or even 24 h/day (3, 13, 42, 47, 48, 78, 81, 97, 106).

**Consequences of IH**

Intermittent hypoxia induces a series of cellular, molecular, and pathophysiological responses that result in either adaptation and survival or injury and cell death. In the adult animal, IH leads to oxidative stress (157, 193) and induces lipid peroxidation (167), increased production of stress responsive proteins (75, 158), and neuronal apoptosis (70, 167, 204). Animal studies have demonstrated executive dysfunction, increased responsiveness to novelty, and locomotor hyperactivity that persists into adulthood (39–41, 166).

**FIGURE 2.** Spectroscopic images of mouse hippocampus and thalamus

A: spectroscopic images of mouse hippocampus and thalamus were obtained before measurements of metabolite levels. The hippocampus (red and green lines) and thalamus (red lines) were outlined manually, and a midline was calculated (blue line). Then voxels were reconstructed along the midline (yellow circles), starting from the most medial position automatically. B: representative spectra from mice exposed to normoxia and 4 wk of chronic intermittent hypoxia (CIH) are shown in this figure. Three dominant resonances are seen in the \(^1\text{H} \) spectrum, N-acetyl aspartate (NAA) (2.0 ppm), creatine (Cr) (3.0 ppm), and choline (Ch) (3.2 ppm). C: graphical representation of the NAA/Cr ratios in the hippocampus and thalamus of mice exposed to normoxia (control); \( n = 5 \), 4 wk of chronic constant hypoxia (CCH; \( n = 5 \)), 4 wk of CIH (\( n = 4 \)), and 4 wk of CIH followed by 4 wk of normoxia [return to normoxia group (CIH-N); \( n = 6 \)]. C, top: hippocampus; \(*\) significant differences between the hippocampi from CIH-exposed mice and those from control \( P < 0.015 \), CIH (\( P = 0.022 \)), and CIH-N \( P = 0.006 \) mice. C, bottom: thalamus; \(*\)NAA/Cr levels significantly different between control and CIH thalamus \( P < 0.017 \); \#significant difference between thalami of CIH and CIH-N mice \( P < 0.015 \).
Indeed, IH causes substantial neurobehavioral deficits (70, 166) and cardiovascular morbidity (for review, see Ref. 145).

**Functional alterations in brain and heart**

Regions of the brain are differentially sensitive to hypoxic or ischemic stress. In general, it is thought that the differential sensitivity of different brain regions is due to several factors, including differences in synaptic input, neurotransmitters released, neurotransmitter receptors, antioxidant capabilities, and signaling pathways. The hippocampus, which is an important site for learning and memory, is also well known to be sensitive to ischemic stress (27, 64, 103), with the CA1 region being particularly sensitive. However, our studies and those of others have shown that brain stem neurons are extraordinarily sensitive to constant hypoxic stress much more than CA1 or cortical cells (46, 89, 143). Indeed, hypoglossal neurons, for example, depolarize within 1–2 min and are in a depolarization block after few additional minutes, whereas CA1 and cortical neurons can last much longer. In humans, brain stem neurons may not seem to be injured during hypoxic stress simply because they are well vascularized, unlike certain areas of the cortex that are not, which makes this region very vulnerable.

The pattern of hypoxic stress is also an important factor that determines vulnerability. For example, it has been found that IH may have a more deleterious impact on brain, heart, and vascular system than a constant stress. The reported pathophysiology of hippocampal impairment sets in after several days of IH compared with much less of an impact by constant hypoxia (67). IH led to impairment in spatial memory as measured by escape latency and swim path length at 7 days of exposure but demonstrated significant recovery at 14 days and even more so at 30 days. These rats also displayed learning deficits in a Morris water maze in that IH induced longer escape latencies and decreased swim path lengths. At a more cellular level, there was lipid peroxidation (210) and glialosis in IH. Furthermore, peak apoptotic staining occurred at 24–48 h, and this declined to control levels by 14 days of exposure, as measured by both terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling (TUNEL) and single-stranded DNA staining, and these changes paralleled the alterations seen in memory and learning. Whether these changes in rodents remind us of the changes that are seen in OSA/H in humans where hippocampal gray matter volumes are decreased remains to be determined (125, 134).

Other studies have demonstrated that IH induces oxidative damage in specific sleep/wake brain regions such as lateral forebrain and posterior/lateral hypothalamus (193). IH produced an increase in inducible NOS (iNOS) activity measured 2 wk after exposure, as well as an increase in a marker of lipid peroxidation, isoprostane 2α, in these regions in wild-type mice but iNOS−/− mice were resistant to oxidative damage (210). This indicates that some of these oxidative changes are mediated by iNOS in IH.

Age is another important consideration when assessing the damage caused by IH, as is illustrated in many publications in the literature (73, 86–88, 110). For example, IH during the neonatal period may have a severe impact on learning and memory in juvenile rats. Some recent studies have demonstrated that alterations in the dopaminergic activity of the prefrontal- striatal circuitry are important in IH-induced memory deficits (41). Sleep-wake architecture also differed between posthypoxic rats exposed during the neonatal period and their controls in that posthypoxic rats exhibited less wakefulness and more REM sleep (39). Additionally, posthypoxic rats demonstrated locomotor hyperactivity and impaired working memory on an eight-arm radial maze. Adult and aged rats also demonstrate IH-induced damage, i.e., increased apoptosis and memory impairment, that increases with age (73).

OSA/H patients often have systemic hypertension and this is mediated by an intact carotid body and pre- ganglionic sympathetic excitation (149). IH in humans leads to a sustained increase in blood pressure that persists beyond the hypoxic period (reviewed in Refs. 59, 140, 151). In OSA/H in humans and IH in animal models, this persistent hypertensive state appears to be due to augmented arterial chemoreceptor activation of the sympathetic nervous system (138). It is interesting to note from animal studies that a central component may indeed exist in that brain stem neurons are activated by IH and contribute to the systemic hypertension.

As to the heart, IH may induce hypertrophy that is similar to that seen in OSA/H patients (30, 56, 111). However, this is not always obtained as much as in constant hypoxia (CH) in animals and humans. CH also induces cardiac hypertrophy in mammals, and the impact of CH is greater than that of IH (33, 133, 135). Our laboratory and others have reported specifically that right ventricular hypertrophy and myocyte hyperplasia occur in response to IH and CH (33, 56, 58). In addition, abnormal myocardial architecture and increased interstitial space is observed in response to IH (121). In rodents, IH can lead to left ventricular global dysfunction and even ventricular remodeling. These functional and anatomical changes in the brain and heart may impose an additional stress on the organism that will adversely affect the aging process.

**Cerebral and myocardial vascular density.** IH induces increased cerebral angiogenesis, as measured by Glut-1 immunoreactivity, but to a lesser extent than continuous hypoxia (13, 105, 106). In a study performed in our laboratory, we demonstrated that IH for 4 wk led to an increase in cerebral vascular density as measured by immunoreactivity of Glut-1 and to a
decrease in myelination as detected by fluoromyelin staining (106). Because the increase in vascular density and decrease in myelination was much greater in constant hypoxia than in IH, we investigated whether there is any reversal of effect only in constant hypoxia. Interestingly, after a return to normoxia for an equivalent period of time, vascular density was reversed to control levels; however, myelination was not restored to control, indicating that, at least in the mouse, the effects of constant hypoxia are not necessarily reversible in the brain. Vascular endothelial growth factor (VEGF) levels are also increased after several weeks of IH but not during constant (or continuous) hypoxia (105, 116). Kalaria and colleagues (105) observed that there was a significant, linear increasing trend in Glut1 immunoreactivity from normoxic to IH to CH ($R^2 = 0.73; P = 0.007$). Additionally, VEGF staining in neurons and some glia was increased in IH but not during CH. Whether there is increased microvascularity and angiogenesis in patients with OSA/H as in IH is unclear at the moment.

In rats exposed to IH, the myocardium demonstrated more capillaries per fiber area and per fiber perimeter and hence increased capillary density (144). These changes were assumed to be an adaptive strategy aimed at restoring efficient $O_2$ delivery to mitochondria of cardiac muscles. Increased angiogenesis during stress is usually not an efficient process and results in abnormal, leaky vessels that promote edema formation. This puts an additional burden on the vasculature and organism that may also impact aging.

**Cerebral metabolites.** In a study of OSA/H patients, it was found that $N$-acetyl-aspartate/creatine (NAA/Cr) compounds were significantly increased, and this was most likely due to a decrease in creatine-containing compounds rather than a change in NAA-containing compounds (15). In this study, decreased levels of creatine were correlated with worse OSA/H severity and neurocognitive performance. On the other hand, our laboratory has demonstrated that there were significant alterations in NAA-to-Cr ratios in the hippocampus and thalamus of mice exposed to IH (47). In studies that we have performed in our laboratory, mice exposed to 4 wk of IH demonstrated significantly decreased NAA-to-CR ratios in the hippocampus and thalamus, whereas those exposed to 2 wk of CH did not show any change in this ratio (FIGURE 2). Decreased NAA-to-CR ratios have been implicated in neuronal mitochondrial dysfunction and injury (95). Intriguingly, NAA-to-CR ratios return to control levels after a return to normoxia for 4 wk in mice exposed to IH for a previous 4 wk. This recovery may be due to a restoration of mitochondrial function in existing neurons or an increase in neurogenesis. Differences in our study and that described above may be due to species and technical differences.

Although we have shown that NAA/Cr decreases in IH and it can be a marker for cell dysfunction or injury, we cannot address at present the exact mechanism(s) that leads to this finding in the brain. Suffice it to say that there are potentially many mechanisms that can play a role in neurons such as ROS-mediated mitochondrial damage and inflammation.

The above-mentioned functional alterations in the CNS and the cardiovascular system result most likely from the persistent activation (or inactivation) of various signaling pathways, namely those involved in NF-$\kappa$B, HIF, and ROS, as well as insulin and glucose metabolism and their cross-talk (163). Part of this is illustrated conceptually in FIGURE 1 and reinforces the concept that aging may be adversely affected by IH-induced damage.

**Ion channel and transporter mechanisms.**

Hypoxia can affect a variety of channels and transporters in the CNS and heart. Often, and especially in anoxia-resistant organisms, the initial response to hypoxia or ischemia is a reduction in membrane channel conductance by altering potassium, sodium, and chloride fluxes. This reduces the energy requirements of neurons during periods of $O_2$ lack and is referred to as “channel arrest.” Turtle neurons are notorious in reducing energy requirements via this mechanism (96). In general, however, mammalian (rodents and humans) neurons increase their input resistance at the very beginning but then increase their conductance and quickly reach a depolarization block, especially in brain stem neurons (46). A number of processes set in the first several minutes of the hypoxic stress.

Over a prolonged period, IH has an impact on neuronal excitability that is age dependent. In neonatal mice exposed to IH ($7.5\% O_2$ 8 h/day for 4 wk starting at P2 or P3), it was observed that IH led to decreased excitability in freshly dissociated hippocampal CA1 neurons (81). IH reduced action potential amplitude and firing frequency in isolated hippocampal CA1 neurons (38), and decreased $Na^+$ channel conductance (96). Again, studies have shown that $Na^+$ channels were not the only ones affected by IH. Indeed, using whole cell patch clamp in isolated NTS.
neurons (38), the authors demonstrated that NMDA currents were reduced, and it was postulated that this was due to IH-induced oxidative stress, as suggested by Prabhakar and colleagues (149, 150). In other studies, Payne et al. (147) exposed adult male Sprague-Dawley rats to IH, and population spike amplitudes were measured in the CA1 region. LTP was induced and was maintained for 15 min in 70% of control slices and reached a population spike amplitude that was >140% of pre-LTP amplitudes. However, a majority of IH-exposed slices failed to sustain LTP and their response was not as remarkable as those of the control slices. The authors suggested from these studies that the electrophysiological properties of the CA1 neurons are disrupted by IH exposure. IH has also been reported to decrease the open probability of large conductance, calcium-activated potassium (BK) channels in the CA1 hippocampal region without affecting the unitary conductance or reversal potential (191).

Activities of ATPases, including the Na+-K+-ATPase, were also affected by IH. For instance, an increase in the activity of Mg2+-ATPase but a decrease in the activities of the Na+-K+-ATPase, high-affinity Ca2+-ATPase, and Ca2+-Mg2+-ATPase activities were found in synaptic membranes (18). Other transporters are also affected by exposure to IH. Acid-base transporters have been shown to play a crucial role in determining neuronal fate during prolonged IH. For example, studies performed in our laboratory have previously shown that acid-base transporters, such as sodium-hydrogen exchanger (NHE-1) and the sodium-bicarbonate co-transporter (NBC), are differentially expressed in various brain regions and that the response to intermittent hypoxia is unique to each brain region such as hippocampus and cerebral cortex (48). IH exposure led to a decrease in the majority of acid-extruding transporters such as NHE-1, NHE-2, and NBCs within the CNS, and this may render neurons less capable of responding to an acidic challenge and thereby suffer injury or death during IH. For example, we have recently demonstrated that inhibition of NHE-1 activity in culture renders neurons more susceptible to hypoxia-induced cell death, but inhibition of HCO3−-sensitive transporters such as NBCs rescues neuronal death during hypoxia (205).

Again, studies of IH in the heart are few. However, binding studies of calcium release channels/ryanodine receptors (RyRs), which play a major role in intracellular Ca2+ handling in cardiac myocytes, were performed in response to IH and CH using [3H]ryanodine binding assay. Maximal binding (Bmax) was decreased in response to CH, which may provide cardioprotection against ensuing ischemic exposure. On the other hand, short-term IH caused no change in Bmax, whereas long-term IH decreased Bmax in rat heart homogenates (196).

Since most electrophysiological studies on neurons, glia, and cardio-myocytes are of short-term duration, most pathways that can lead to cell injury and decreased life span are related to specific ion flux changes such as Na+, Ca2+, and pH, and energy metabolism and loss of ATP synthesis. These fluxes are, by and large, often affected by changes in redox states. Ion channels and a variety of transporters maintain metabolic homeostasis in cells, and changes in their expression or activity could lead to imbalances that render cells more prone to oxidative stress and senescence.

“Hypoxia has effects on cellular differentiation in neuronal precursors that appear to be mediated by the Notch signaling pathway.”

Neurotransmitter and neuromodulatory systems

Hypoxia has been shown to activate several neurotransmitter or neuromodulatory systems such as glutamate, GABA, adenosine, and opiates (162, 187, 197). Dopamine neurotransmission is reduced in subcortical structures during IH, and this leads to decreased wakefulness (41). Furthermore, extracellular levels of dopamine are decreased in these animals, indicating impaired dopaminergic function (40). Long-term IH also reduced tyrosine hydroxylase activity but not protein expression in the CNS (77), whereas 2 wk of IH exposure led to a decrease in choline acetyltransferase-positive cells in the CNS (165).

Fanous et al. (57) demonstrated that NMDA receptor subunit 1 (NR1) mRNA was significantly increased in several brain stem nuclei such as the nucleus tractus solitarius (NTS) and inferior olivary nucleus (ION), but NR1 protein was only increased in the ION after acute IH with hypercapnia in piglets (57). In other studies where IH occurred over 2–4 days, there was a more modest increase in NR1 expression compared with the above study (126), and these were seen in a more restricted group of brain stem nuclei, namely the hypoglossal nucleus, dorsal motor nucleus of the vagus, and gracile nucleus. Pieris et al. (148) also demonstrated increased brain-derived nerve growth factor (BDNF) expression in brain stem. Longer-term IH studies induced decreased responsiveness of hypoglossal motoneurons to serotoninergic and excitatory amino acid (NMDA) stimulation as well as increased lipid peroxidation in dorso-medial medulla. The reduced responsiveness of hypoglossal motoneurons and increased lipid peroxidation were abrogated by a SOD-mimetic, tempol, provided in the drinking water. The authors conclude that IH impairs hypoglossal nerve responsiveness to serotonin (5-HT) and NMDA through a redox-mediated phenomenon (194). Interestingly, these data differ substantially from those of studies using a short-term IH paradigm (11) where...
is generated not only from conditions of increased O2 availability of O2. This ultimately leads to increased free-radical production as detected by electron paramagnetic resonance spectroscopy (ESR/EPR), and a decrease in reduced cysteine (199). Oxidant stress increases under hypoxia can activate multiple signaling pathways including HIF-1α protein stabilization and gene regulation (172).

Another radical that is produced during hypoxia/reoxygenation is nitric oxide (NO-), which is derived from arginine by the enzyme nitric oxide synthase (NOS). There are three isoforms of the enzyme including constitutive neuronal NOS-1 (nNOS), endothelial NOS-3 (eNOS), and the inducible NOS-2 (iNOS) (54). It is postulated that NO- generated by NOS during hypoxia can react with superoxide to produce peroxynitrite and precipitate membranal and cellular damage.

Antioxidant defenses include enzymatic (catalase, endoperoxidases, and dismutases) and non-enzymatic (glutathione, cholesterol, ascorbic acid, and tocopherol) mechanisms [see review from Mishra and Delivoria-Papadopoulos (131)]. Hypoxic stress may also lead to impairment of anti-oxidant defense mechanisms by alterations in the activities of glutathione reductase and glutathione peroxidase (19), which can impair mitochondrial activity at complexes I and III (14). ROS inactivate antioxidant molecules (211) and other free-radical quenchers and anti-oxidant precursors have been shown to enhance cell viability in hypoxic or ischemic stress (10).

Investigators interested in the cardiorespiratory responses to IH have uncovered regions of the brain stem that are modulated by this exposure. Griffioen et al. (80) provide evidence for the involvement of ROS in acute IH-induced inspiratory-evoked excitatory neurotransmission to cardio-inhibitory vagal neurons in the nucleus ambiguous (NA). Thick in vitro brain stem slices obtained from newborn rats were exposed to intermittently short periods of hypoxia. Postsynaptic glutamatergic neurotransmission was measured in parasympathetic cardioinhibitory neurons identified by fluorescent tracer using whole cell patch clamp. ROS was assayed using 2′,7′-dichlorofluorescein (DCF), a fluorophore that is excited by ROS. IH did not block both excitatory and inhibitory binding of nNOS protein in mice (123). N-acyetyl-cysteine, an anti-oxidant, has been reported to have neuroprotective effects on measures of injury or dysfunction that affects the outcome such as lipid peroxidation in tissues that result from ROS and Ca2+ levels. Changes in neurotransmitter and ion channel activity can lead to impaired homeostasis that can increase oxidative stress, which is believed to be the major component of neurodegenerative disorders such as Alzheimer’s and Parkinson’s diseases (16, 178). Injury normally results when the anti-oxidant capacity of the cell is overwhelmed. Even though it is still a controversial issue, it is important to realize from recent evidence that ROS is continuously generated by cells, and the toxic capability of ROS at high levels has been well documented (22, 60). Cellular sources of ROS production include the mitochondrial electron transport chain, NADPH oxidases, hypoxanthine/xanthine oxidase, and cytochrome P450 of the endoplasmic reticulum (reviewed in Refs. 9, 24, 112). Whether most ROS that is produced in cells as a result of OSA/H comes from mitochondria is not clear at present.

It has been known that aging and its neurocognitive and pathological sequelae are correlated with the accumulation of ROS (theory of aging) (7, 49, 128). In addition, ROS have been implicated in several neurodegenerative disorders such as Alzheimer’s and Parkinson’s diseases (16, 178). Injury normally results when the anti-oxidant capacity of the cell is overwhelmed. Even though it is still a controversial issue, it is important to realize from recent evidence that ROS is generated not only from conditions of increased O2 such as in hypoxia but also from hypoxia (31, 37, 84, 152, 156). Hypoxia paradoxically stimulates the production of ROS from mitochondria, and these species can then modulate transcriptional and posttranslational responses to low oxygen (50, 175). Hypoxia per se is able to generate increased ROS due to the lower availability of O2 to act as an electron sink/acceptor that allows excess electrons to form superoxide with available O2. This ultimately leads to increased hydroxyl and peroxynitrite radicals (2, 37, 200, 202). It is believed now that the most likely site for ROS generation during hypoxia is at complex I and complex III of the mitochondrial electron transport chain. Experimental evidence for increased ROS during hypoxia include oxidation of fluorescent probes, decreases in the anti-oxidant reduced glutathione, increased free-radical production as detected by electron paramagnetic resonance spectroscopy (ESR/EPR), and a decrease in reduced cysteine (199). Oxidant stress increases under hypoxia can activate multiple signaling pathways including HIF-1α protein stabilization and gene regulation (172).

Although the relation between NMDA and Ca2+ metabolism has been well studied, it is the balance between excitatory and inhibitory neurotransmission that affects the outcome such as lipid peroxidation in tissues that result from ROS and Ca2+ levels. Changes in neurotransmitter and ion channel activity can lead to impaired homeostasis that can increase oxidative stress, which is believed to be the major component of neurodegenerative aging.

**Oxidative consequences of IH**

Patients who suffer from sleep-disordered breathing states demonstrate increased systemic markers of oxidative stress (26, 119, 186). Free-radical generation has been reported to be increased in OSA/H patients (12, 71, 109, 120, 170, 174). Free radicals such as superoxide, hydroxyl and peroxynitrite (ONOO−) are continuously generated by cells, and the toxic capability of ROS at high levels has been well documented (22, 60). Cellular sources of ROS production include the mitochondrial electron transport chain, NADPH oxidases, hypoxanthine/xanthine oxidase, and cytochrome P450 of the endoplasmic reticulum (reviewed in Refs. 9, 24, 112). Whether most ROS that is produced in cells as a result of OSA/H comes from mitochondria is not clear at present.

It has been known that aging and its neurocognitive and pathological sequelae are correlated with the accumulation of ROS (theory of aging) (7, 49, 128). In addition, ROS have been implicated in several neurodegenerative disorders such as Alzheimer’s and Parkinson’s diseases (16, 178). Injury normally results when the anti-oxidant capacity of the cell is overwhelmed. Even though it is still a controversial issue, it is important to realize from recent evidence that ROS is generated not only from conditions of increased O2 such as in hypoxia but also from hypoxia (31, 37, 84, 152, 156). Hypoxia paradoxically stimulates the production of ROS from mitochondria, and these species can then modulate transcriptional and posttranslational responses to low oxygen (50, 175). Hypoxia per se is able to generate increased ROS due to the lower availability of O2 to act as an electron sink/acceptor that allows excess electrons to form superoxide with available O2. This ultimately leads to increased hydroxyl and peroxynitrite radicals (2, 37, 200, 202). It is believed now that the most likely site for ROS generation during hypoxia is at complex I and complex III of the mitochondrial electron transport chain. Experimental evidence for increased ROS during hypoxia include oxidation of fluorescent probes, decreases in the anti-oxidant reduced glutathione, increased free-radical production as detected by electron paramagnetic resonance spectroscopy (ESR/EPR), and a decrease in reduced cysteine (199). Oxidant stress increases under hypoxia can activate multiple signaling pathways including HIF-1α protein stabilization and gene regulation (172).

Another radical that is produced during hypoxia/reoxygenation is nitric oxide (NO-), which is derived from arginine by the enzyme nitric oxide synthase (NOS). There are three isoforms of the enzyme including constitutive neuronal NOS-1 (nNOS), endothelial NOS-3 (eNOS), and the inducible NOS-2 (iNOS) (54). It is postulated that NO- generated by NOS during hypoxia can react with superoxide to produce peroxynitrite and precipitate membranal and cellular damage.

Antioxidant defenses include enzymatic (catalase, endoperoxidases, and dismutases) and non-enzymatic (glutathione, cholesterol, ascorbic acid, and tocopherol) mechanisms [see review from Mishra and Delivoria-Papadopoulos (131)]. Hypoxic stress may also lead to impairment of anti-oxidant defense mechanisms by alterations in the activities of glutathione reductase and glutathione peroxidase (19), which can impair mitochondrial activity at complexes I and III (14). ROS inactivate antioxidant molecules (211) and other free-radical quenchers and anti-oxidant precursors have been shown to enhance cell viability in hypoxic or ischemic stress (10).

Investigators interested in the cardiorespiratory responses to IH have uncovered regions of the brain stem that are modulated by this exposure. Griffioen et al. (80) provide evidence for the involvement of ROS in acute IH-induced inspiratory-evoked excitatory neurotransmission to cardio-inhibitory vagal neurons in the nucleus ambiguous (NA). Thick in vitro brain stem slices obtained from newborn rats were exposed to intermittently short periods of hypoxia. Postsynaptic glutamatergic neurotransmission was measured in parasympathetic cardioinhibitory neurons identified by fluorescent tracer using whole cell patch clamp. ROS was assayed using 2′,7′-dichlorofluorescein (DCF), a fluorophore that is excited by ROS. IH did not block both excitatory and inhibitory binding of nNOS protein in mice (123). N-acyetyl-cysteine, an anti-oxidant, has been reported to have neuroprotective effects on measures of injury or dysfunction that affects the outcome such as lipid peroxidation in tissues that result from ROS and Ca2+ levels. Changes in neurotransmitter and ion channel activity can lead to impaired homeostasis that can increase oxidative stress, which is believed to be the major component of neurodegenerative aging.
also blocked the increase in ROS in VLM cells. Therefore, the authors postulated that, since these ventrolateral cells are located in an area known to contain neurons of respiratory rhythm control and temporal blocked both the electrophysiological and ROS response to IH, they may be the source of excitatory neurotransmission to the cardio-inhibitory vagal cells in the NA and that excitatory neurotransmission at the NA vagal cells was modulated by ROS. This inhibition is thought to provide cardio-protection in response to IH by decreasing energy demands on the myocardium during reduced $O_2$ availability.

Other studies of IH also demonstrate significant oxidant stress (167). IH increases iNOS expression in a temporally defined manner and leads to increased peroxynitrite formation, which may underlie IH-induced neuronal injury and spatial memory deficits in mice (123). In another study, male rats were exposed to either constant hypoxia or IH (10%) for half a day. It was demonstrated that IH, but not CH, increased iNOS protein expression but not eNOS and nNOS protein expression in rat cortex. IH also induced increases in iNOS mRNA and activity. iNOS mRNA was increased early on and remained elevated after 2 wk, whereas iNOS activity declined after an early increase. Similarly, IH induced increases in nitrotyrosine, nitrate, and nitrite in rat brain, with a similar temporal profile.

Oxidative stress and oxidant signaling are evident in the cardiac tissue of patients with OSA/H and cardiovascular diseases [reviewed by Suzuki et al. (186)]. IH induces cardiac oxidative stress (29, 146) as well as myocyte hypertrophy and apoptosis in cultured cardiomyocytes (169). Heart mitochondrial NOS (mtNOS) activity increases during acute and chronic hypoxia (114, 192). Increased $NO^\cdot$ production inhibits mitochondrial respiration and promotes the formation of superoxide and $H_2O_2$ by the reversible and $O_2$-competitive binding of $NO^\cdot$ to cytochrome oxidase (20, 32). Superoxide and $H_2O_2$ can act on cytosolic regulatory proteins such as mitogen-activated protein kinases that are involved in the cell cycle and apoptosis. NO- and other inhibitors of mitochondrial respiration lead to HIF1α de-stabilization by providing increased cytosolic $O_2$ and allow for activation of prolyl hydroxylases and HIF1α degradation. IH can also lead to modulation of NADPH oxidase (NOX) activity and expression in the heart (94). Whereas wild-type mice in IH demonstrated significant oxidative stress including increased superoxide, mice deficient in gp91 (NOX2) did not, implying that ROS play a significant role on the pathophysiology of IH.

Even though controversial, ROS is also believed to be involved in age-related alterations in the cardiovascular system (reviewed in Refs. 65, 115, 179). There is evidence that systemic oxidative stress, measured as an increase in lipid peroxidation products such as thiobarbituric acid reactive substances (TBARS), is directly correlated with age (28, 130). It is also noteworthy that anti-oxidant levels appear to decrease with advancing age [for review, see Junqueira et al. (102)].

Even though cardiac mtNOS activity declines with age (34), it is possible that free radical damage associated with ONOO$^-$ during hypoxia would contribute to decreasing lifespan. It was shown that Wistar rats living in hypobaric hypoxia had decreased lifespans compared with controls (192). Hypoxic rats died at the age of 79–92 wk, whereas control rats lived longer than 92 wk. This indicates that chronic exposure to moderate hypoxia can negatively influence the aging process. It is therefore possible that if IH does indeed lead to increased oxidative stress via increased ROS or decreased anti-oxidant pools for which there is growing evidence and consensus, IH may also negatively impact the aging process and lead to a decrease in lifespan.

**Apoptosis and proliferation**

IH leads to apoptosis in both neonatal and adult animals, and the extent depends on brain region (66, 70, 74). The impact of development on the susceptibility to IH was examined in neonatal rats exposed to IH for 48 h at various ages in early life (76). Assays for apoptosis included both TUNEL staining and an antibody to single-stranded DNA (ssDNA) (61). IH significantly increased the number of TUNEL-positive cells in the cortex and CA1 region of the hippocampus at all ages, with a smaller number of apoptotic cells at P2 and P5. In contrast, peak apoptosis occurred at P10–P25, when compared with both younger and older animals. Similar results were obtained using ssDNA staining and immunofluorescent analysis for cleaved caspase 3 (FIGURE 3). However, no differences were evident in cortex and CA1 at any developmental stage for both approaches.

In rats exposed to a similar pattern of IH, an increase in apoptosis was also noted in cortex and CA1 region of the hippocampus (70). There were marked increases in apoptotic staining detected by TUNEL and ssDNA staining in the CA1 region but not CA3 region of the hippocampus and in the cerebral cortex. It was also noted that IH disrupted the neuronal cytoarchitecture in CA1 and cortex as detected by NR1 and cfos staining, which was also increased in expression.

Although the CA1 region of the hippocampus is susceptible to injury during IH exposure, the CA3 region is relatively resistant (70). Proteomic analysis of proteins in the CA1 and CA3 was performed to detect differences in basal expression in these two regions as well as in response to IH (75). Cytoskeletal- and metabolism-related proteins were found to be more abundant in normoxic CA1 than in CA3, and this may be due to the greater cellularity of the CA1 region. IH induced the upregulation of 32 proteins in CA1 but only 7 in CA3. In the CA1, these proteins fell into three categories, cytoskeleton-related proteins such as $\alpha$- and $\beta$-tubulin, metabolism-related proteins such as ATP synthase, and apoptosis-related
proteins such as stress-inducible chaperones (hsp70). The authors postulated that survival mechanisms are initiated in the early stages of IH, as evidenced by the increase in anti-apoptotic and metabolic proteins, that are eventually overwhelmed by the continued stress of IH that ultimately leads to peak apoptosis at 48 h of IH in the more vulnerable CA1 region of the hippocampus (70).

IH induces apoptosis in rat myocardium as shown by increased TUNEL-positive cell number and caspase 3 activity after 6 wk of IH (30). Animals subjected to IH show increased infarct size after total global ischemia/reperfusion in IH hearts compared with those from normoxic or control hearts (101). Hence, this study purports that rat hearts exposed to long-term IH are more susceptible to ischemia, unlike the idea of preconditioning (45, 215), indicating that the paradigm used can affect the process leading to preconditioning. For example, studies from our laboratory have demonstrated that previous exposure to long-term moderate hypoxia renders neocortical neurons more vulnerable to a subsequent acute stress such as O₂ deprivation. Exposed brain slices depolarized sooner and recovered more slowly than control slices from acute in vitro hypoxia (142). It therefore becomes important to delineate the paradigm of preconditioning used and its applicability to specific cells and tissues.

Cell injury and apoptosis are not limited to brain and heart in IH but occur also in the cerebral and coronary arteries, which initiate and propagate further cell death and remodeling in the lumen of these blood vessels. It is often the remodeling of vessels that leads to additional stress, and a vicious cycle sets in. It is possible that oxidant stress and inflammatory mediators modulate the intermittent hypoxia-induced vascular dysfunction (105, 171, 189). Vascular dysfunction is central to our hypothesis that IH-induced alterations can negatively affect aging.

Stem cells and Notch signaling

In contrast to cell death that can occur during IH, there seems to be an increase in neuronal replication in certain regions of the CNS. Neurogenesis continues
throughout life in the mammalian brain, specifically within the subventricular zone (SVZ) around the lateral ventricles and in the subgranular zone of the dentate gyrus (5, 36, 53, 62, 68, 69, 93, 154). Even though most neurogenesis occurs prenatally, neurogenesis continues postnatally in the hippocampus (53) and olfactory bulb (108, 124) and has recently been postulated to occur in the cerebral cortex and spinal cord as well (127, 206). It has been demonstrated that neurogenesis also occurs in the adult brain and in the postnatal neonate in response to injury (127, 182). Moreover, it has been recently suggested that neurogenesis in the CNS can occur in response to hypoxia-induced injury. For example, severe hypoxia for a short period results in apoptotic cell death in the CA1 region of P1 rat hippocampus as assayed by Bcl-2, Bax, and caspase 3. However, after a return to normoxia, rats demonstrated significant recovery of neuronal numbers via neurogenesis by P21 (36). In a corresponding experiment where neonatal mice were subjected to sublethal hypoxia (9.5–10.5% O2), Fagel et al. (55) also observed neuronal cell loss that was restored after a return to normoxia by increased proliferation of astrocytes, oligodendrocytes and, importantly, neurons. A mechanism by which this might occur could be the hypoxia-induced upregulation of fibroblast growth factor (FGF) signaling that promotes neocortical neurogenesis (63).

Even though there are only a few published reports on this topic, IH seems also to induce the proliferation and differentiation of neural stem cells. In a study by Zhu et al., it was reported that an intermittent hypoxia paradigm of moderate hypoxia for 2 weeks led to increased bromo-deoxyuridine (BrdU) incorporation in the subventricular zone (SVZ) and dentate gyrus in presumably young adult rats (214). Interestingly, although the number of dividing cells returned to control levels in the SVZ after 4 wk posthypoxia, the numbers of dividing cells doubled in the DG, but these cells apparently did not differentiate into neurons. The authors therefore concluded that neural precursors in the SVZ and DG respond differently to hypoxia, as has been reported in the case of transient global ischemia in the gerbil (186). In another study using IH, neurogenesis was found to rebound later after IH. Using nestin and neurofilament markers, IH initially induced a reduction in BrdU-positive cells at a few days; however, this was followed by an increase in BrdU-positive cell numbers after a few weeks (72). Of note, cyclic-AMP response element binding protein (CREB) is important for induction of neuronal replication and was also demonstrated to be decreased early in response to IH but also reversed that trend and increased. These events occurred without any demonstrable changes in proteins involved in synaptogenesis such as synaptophysin, syntaxin, 25K synaptosome-associated protein (SNAP25), vesicle-associated membrane protein (VAMP)/synaptobrevin, or drerin.

Hypoxia has effects on cellular differentiation in neuronal precursors that appear to be mediated by the Notch signaling pathway. It has been recently shown that HIF-1α binds to the Notch intracellular domain, and this complex can bind to Notch-responsive promoters and activate Notch-dependent genes (83). This phenomenon underlies the ability of stem cells to remain in the undifferentiated state during normoxia and to replicate and differentiate under hypoxic conditions. However, neurogenesis is usually inadequate and does not serve to totally replenish cells that have been lost during injury. Therefore, cell loss in brain and heart during IH may impact cellular function and aid in the progression of aging.

Gene transcription

Even though gene transcription is generally supposed to be repressed during severe hypoxia (118), there are certain genetic programs that are upregulated, especially the universal hypoxic regulator hypoxia inducible factor 1 (HIF-1), which is central to the response of tissues to hypoxia (195). HIFs are members of the basic helix-loop-helix, Per/ARNT/Sim (HLH-PAS) protein family and consist of three O2-regulated alpha chains (HIF-1α, -2α, and -3α) and a constitutive β-chain [HIF-1β, aryl hydrocarbon receptor nuclear translocator (ARNT)] (129, 161). HIF-1α was first identified by Wang and Semenza (195), and the HIF-1α homolog HIF-2α, originally termed endothelial PAS protein 1 (EPAS-1), which shares similar functional and regulatory features but has different roles, was later described (201).

HIF-1α is unstable under normoxic conditions due to the action of prolyl hydroxylases (PHD1, 2, and 3), which predispose to its ubiquitination by the E3 ligase complex that includes the von Hippel-Lindau tumor suppressor protein (pVHL) and degradation by the proteasome (104). Under normoxic conditions, the pVHL binds to the oxygen-dependent degradation domain (ODD) in the carboxy terminus of HIF-1α and hydroxylates prolines 402 and 564, which allow poly-ubiquitination of HIF-1α. Additionally, transactivation of HIF-1 by p300 is prevented by another hydroxylase, the asparaginyl hydroxylase or factor-inhibiting HIF-1 (FIH-1), which represses activity of HIF1 (21). These hydroxylases require molecular O2 as a substrate and oxoglutarate as a co-substrate as well as iron (Fe2+) to hydroxylate these specific proline residues. Therefore, in the absence of O2 or during iron depletion as with desferoxamine, HIF-1α can no longer be hydroxylated and degraded and now accumulates within cells. Hypoxia leads to a widespread accumulation of HIF-1α in virtually all tissues with subsequent tissue-specific target gene activation (185). HIF-1α and its target genes are also upregulated in the penumbra of brain infarcts (1).

Upon dimerizing of HIF-1α with HIF-1β, HIF-1 translocates to the nucleus. HIF-1 binds a consensus sequence within the hypoxia response element (HRE)
in the promoter region of O₂-responsive genes and recruits the co-activator, acetyltransferase CBP/p300 (8). This transcriptional complex regulates the expression of more than 100 pro-survival genes including erythropoietin (EPO), VEGF, and glycolytic enzymes. HIF-1 target genes play essential roles in development, angiogenesis, erythropoiesis, glucose transport, glycolysis, iron transport, and cell proliferation/survival. Neuroprotection provided by HIF-1 appears to be mediated by upregulation of the EPO gene that

**FIGURE 4.** Alteration in gene expression and protein level of eukaryotic translation initiation factors (eIFs) after chronic hypoxia treatment

A: Profiles of gene expression and regulation of eIFs in 4 individual mice subjected to normoxia (N1-N4), CCH (C1-C4), and CIH (I1-I4) for 1, 2, or 4 wk. Each value is represented by a colored square. Duration of the treatment is indicated before the letter of treatment (e.g., 1I2 = 1 wk CIH, 2nd mouse), whereas the green/red color of the square shows down/upregulation, with brighter colors for higher regulation. Note both the variability and the reproducible pattern among the mice subjected to the same treatment. Note also the darker colors of the normoxic values, since they were closer to the average used in normalization. B: Western blot analysis of eIF-2α and eIF-4E in CCH, CIH, and age-matched NC. Results were reproduced in 3 independent experiments and averaged. C and D: Statistical analysis (t-test) of densitometric analyses of Western results of eIF-2α and eIF-4E. The y-axis depicts the relative protein expression level as a ratio of the protein to its HSC70 density per 40 μg of total protein. Values are means ± SD (n = 3).
reduces the extent of apoptosis via crosstalk between the Jak-2 and NF-kB signaling pathways (43). Studies indicate that HIF-1 induces VEGF expression under hypoxic stress, which can lead to increased paracellular permeability (51). However, severe hypoxia can lead to HIF-1-mediated apoptotic cell death (which can be either adaptive or pathological) that requires p53 (25, 91, 160). Nitric oxide can be induced during hypoxia and may serve a neuroprotective function by stabilizing HIF-1α in addition to acting as a potent vasodilator (90). VEGF levels are also increased after 8 wk of IH but not during CH (105). HIF accumulates in the cytoplasm of cells in the cerebral cortex of old rats exposed to protracted IH (160).

Another transcription factor that is upregulated during IH is the immediate early gene, cfos, which is a component of the activator protein 1 (AP-1) (79, 177). Sica et al (177) examined the expression of cfos, which is used as a marker of neuronal excitation, in the neocortex in response to IH. Rats chronically exposed to IH had a persistent increase in cfos expression in viscerolimbic regions of the cerebral cortex including the medial bank of the prefrontal cortex, infralimbic region of the cingulate cortex, retrosplenial granular cortex, piriform cortex, and lateral temporal cortex. Having previously reported that IH induced cfos in NTS neurons in the brain stem, the authors believe that IH-induced cardio-respiratory activation is initiated at the level of the caudal and rostral medulla and ascend via parallel pathways to the forebrain to provide long-term adaptation to chemo- and baro-receptor responses to IH. However, the evidence that IH induces long-lasting hypertension indicates that there may be a dysregulation of cortical dampening mechanisms acting to attenuate blood pressure increases during extended IH.

In microarray studies performed in our laboratory, mice were exposed to 2 wk of either CH or IH and examined for changes in global gene expression in the CNS (213). The expression level of 80 genes was significantly altered by IH exposure, and 137 genes were altered by CH in the cortex. The sIRNA-mediated knockdown of one gene, sarcospan, that was downregulated in both paradigms, also increased cell death in hypoxia in a cell culture system. IH also has been demonstrated to induce HIF-1α in the heart (23) and in PC12 cells (289). Microarray analysis of myocardium from IH- and CH-exposed mice revealed differential alterations in gene expression (56). Interestingly, eukaryotic translation initiation factor 4E (eIF-4e) was upregulated in CH hearts but downregulated in IH hearts, which may explain the hypertrophic response observed in CH hearts (Figure 4). Also, the downregulation of heart development-related genes such as Notch gene homolog 1 and MAD homolog 4 and the upregulation of proteolytic genes such as calpain-5 may underlie the lack of hypertrophy seen in these studies. Even though changes in gene transcription during IH may serve a protective function, maladaptive transcriptional changes may serve to undermine homeostasis and promote aging.

Summary and Potential for Therapeutic Targets

It is clear from the work presented in this review that 1) the regulation of O2 is important for cell and tissue integrity and 2) when O2 levels are abnormal, especially with intermittent conditions and pathophysiology, cells in specific organs such as brain, heart, liver, and kidney and in blood vessels get injured with pathology that sets a vicious cycle and that begets further pathology. Therefore, disease states that start such an abnormal regulation of O2 can set off cascades that promote premature aging of blood vessels, cells, and organs. It is interesting to note, at least from preliminary work in our laboratory and those of others, that some alterations that occur as a result of hypoxia may not be reversible in the brain (or in other organs). Such data would argue that hypoxia resulting from such disease states as OSA/H ought to be treated to not induce irreversible changes, especially since it pertains to growth and development in early life.

Results from various laboratories have made great progress in the last decade and have opened the way to potential translational targets. For example, OSA/H and intermittent hypoxia have been demonstrated to alter metabolism. If such metabolic effects lead to increased insulin resistance and diabetes, we would draw from these findings a few conclusions. First, individuals suffering from such conditions would be predisposed to premature aging if these metabolic abnormalities are not treated and persist. Second, these are potentially treatable and can be eliminated. Research in this area has made headway in our understanding of disease and importantly is leading to therapeutic measures.

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References


190. Tian X, Zhou D, Wang J, Zapala MA, Xue J, Schork NJ. Process Plan: VP.MultiPage.PDFef4d5184d2e9ec40b3c3caa8ad1dad2c
