Published in final edited form as:

Compr Physiol. 2015 March 1; 5(2): 561–577. doi:10.1002/cphy.c140039.

Peripheral Chemoreception and Arterial Pressure Responses to Intermittent Hypoxia

Nanduri R. Prabhakar^{1,*}, Ying-Jie Peng¹, Ganesh K. Kumar¹, and Jayasri Nanduri¹

Jayasri Nanduri: nanduri@uchicago.edu

¹Institute for Integrative Physiology and Center for Systems Biology for O₂ Sensing, Biological Sciences Division, University of Chicago, Illinois, USA

Abstract

Carotid bodies are the principal peripheral chemoreceptors for detecting changes in arterial blood oxygen levels, and the resulting chemoreflex is a potent regulator of blood pressure. Recurrent apnea with intermittent hypoxia (IH) is a major clinical problem in adult humans and infants born preterm. Adult patients with recurrent apnea exhibit heightened sympathetic nerve activity and hypertension. Adults born preterm are predisposed to early onset of hypertension. Available evidence suggests that carotid body chemoreflex contributes to hypertension caused by IH in both adults and neonates. Experimental models of IH provided important insights into cellular and molecular mechanisms underlying carotid body chemoreflex-mediated hypertension. This article provides a comprehensive appraisal of how IH affects carotid body function, underlying cellular, molecular, and epigenetic mechanisms, and the contribution of chemoreflex to the hypertension.

Introduction

Seminal studies by Fernando de Castro (32) and Cornielle F. Heymans (73) demonstrated that carotid bodies, which are located at the bifurcation of common carotid arteries are the sensory organs for detecting changes in arterial blood oxygen (O₂) levels, and the resulting reflex is a potent regulator of blood pressure. Heymans (74) further suggested that reflexes from aortic region might subserve a similar function. Subsequent studies by Julius H. Comroe Jr. established that reflexes arising from the aortic bodies, which are located in the aortic arch also regulate cardio-respiratory functions during hypoxia (26). Thus, carotid and aortic bodies are regarded as the major peripheral chemoreceptors for detecting arterial blood O₂ levels and the ensuing reflexes maintain cardio-respiratory homeostasis during hypoxia. Tissues with a similar morphology to carotid and aortic bodies have also been described in thorax and abdomen, which are called “paraganglion,” and might serve as additional chemoreceptors (34, 44).

Amongst the peripheral chemoreceptors identified thus far, carotid bodies are the best studied with respect to the mechanisms of O₂ sensing and chemoreflex regulation of cardio-respiratory functions. Carotid body receives sensory innervation from a branch of the

glossopharyngeal nerve called the “carotid sinus nerve” (CSN). Hypoxia increases the CSN activity, which in turn reflexly stimulates breathing, sympathetic nerve activity and increases blood pressure. Earlier reviews on the mechanisms of O₂ sensing by the carotid body and the chemoreflex regulation of cardio-respiratory functions can be found in the articles by Fidone & Gonzalez (48), and Fitzgerald & Lahiri (49). A contemporary and comprehensive analysis of the carotid body and the chemoreflex function in health and disease is presented in a recent review article in the *Comprehensive Physiology* (99).

Hypoxia, which is the primary stimulus to the carotid body, can be continuous such as that occurs during sojourn at high altitude or can be intermittent as experienced by those with sleep-disordered breathing with apnea (i.e., brief cessation of breathing). People with recurrent apnea exhibit several comorbidities including hypertension. A substantial body of evidence suggests that the carotid body chemoreflex contributes to hypertension caused by intermittent hypoxia (IH). Recent studies on experimental models of IH have provided important insights into cellular and molecular mechanisms underlying carotid body chemoreflex-mediated hypertension. The purpose of this article is to provide a comprehensive appraisal of how IH affects the carotid body function, underlying mechanisms, and the contribution of the chemoreflex to the hypertension.

IH and Sleep-Disordered Breathing with Apnea

IH is a hallmark manifestation of sleep-disordered breathing (SDB) with recurrent apnea, which is characterized by transient, repetitive cessations of breathing either due to obstruction of the upper airway (Obstructive Sleep Apnea, OSA) or defective generation of respiratory rhythm by the central nervous system (CNS) (central apnea) (133, 175). In severely affected patients arterial blood saturations can be reduced to as low as 50%. Epidemiological studies showed that recurrent apnea is prevalent in nearly 5% of adult males and 2% of women after menopause. Apnea results in periodic hypoxemia (i.e., IH) and intermittent hypercapnia (i.e., elevated arterial CO₂). A major advance in the field of apnea research is the demonstration that exposing experimental animals to IH alone is sufficient to produce many of the comorbidities including hypertension (51).

IH and Hypertension

Clinical findings

OSA patients exhibit pronounced increases in blood pressure during apneic episodes and elevated blood pressures during daytime even in the absence of apneas (155, 191). OSA is particularly common in patients with resistant hypertension (43). Peppard and co-workers demonstrated a clear correlation between the severity of apnea and subsequent development of hypertension independent of other comorbidities and confounding factors (155). Thus, IH is a major factor contributing to hypertension in OSA patients.

Continuous positive airway pressure (CPAP) is the treatment of choice for OSA. However, the effectiveness of CPAP treatment in controlling hypertension in OSA patients is controversial. For instance, Dudenbostel and Calhoun (43) reported that CPAP treatment is ineffective in normalizing blood pressure in OSA patients with resistant hypertension. On

the other hand, several others reported that CPAP treatment is effective in lowering blood pressure in OSA patients (8, 82, 181). Taken together, these studies demonstrate that recurrent apnea patients exhibit exaggerated blood pressure elevations during apneic episodes and hypertension during daytime.

Studies on animal models

The English bulldog exhibits apneas during REM sleep (69) and obese pigs also show mild SDB with apnea (114, 194). However, whether apnea in these animal models also leads to hypertension is not known. Fletcher (50) developed a rat model of IH, mimicking O₂ saturation profiles seen in recurrent apnea patients. Rats exposed to IH for 30 days develop hypertension (+11 mmHg) recapitulating the phenotype reported in recurrent apnea patients (50). Subsequent studies have confirmed that rodents exposed to prolonged IH do indeed exhibit hypertension, but the magnitude and the onset of hypertension seem to depend on the paradigm of IH (Table 1).

IH and Sympathetic Nerve Activity

Clinical findings

A growing body of evidence suggests that augmented sympathetic nerve activity is a major contributor to the hypertension in OSA patients. Muscle sympathetic nerve activity (MSNA) is reflective of systemic vascular resistance. Consequently, several investigators recorded MSNA in sleep apnea patients (14,67,110,181). Normal subjects without apneas exhibit low levels of MSNA during sleep (78, 136, 182); whereas sleep apnea patients display striking absence of reduced MSNA during sleep (131). Sympathetic nerve activity and blood pressure increase progressively with each episode of apnea and the magnitude of increase is more pronounced during REM (rapid eye movement) sleep, wherein the duration of apneas are longer than in non-REM sleep (131). OSA patients exhibit elevated MSNA during daytime despite the absence of apneas and normal arterial blood gases (131). Exposing normal subjects to a daily regimen of IH for 10 days alone was sufficient to augment MSNA (115). Acute IH akin to apneas leads to long-lasting sympathetic activation in normal subjects (30, 126, 195), which might explain why sleep apnea patients exhibit increased sympathetic nerve activity during daytime. Elevated sympathetic nerve activity in OSA subjects is independent of obesity, which is a common comorbidity in these patients (131). OSA patients exhibit elevated circulating and urinary catecholamines (both norepinephrine and epinephrine), which were attributed to increased sympathetic nerve activity (14, 54, 55, 120, 181). CPAP treatment normalizes sympathetic nerve activity in OSA patients (8, 82, 181). Collectively, these studies suggest that hypertension in recurrent apnea patients is associated with overactive sympathetic nervous system. However, endothelial dysfunction leading to altered vascular reactivity caused by IH is also proposed to contribute to systemic hypertension associated with OSA (4, 89).

Studies on animal models

IH-exposed rats show elevated cervical (60), renal (79), splanchnic (40), thoracic (204), and lumbar (119) sympathetic nerve activity. Sympathetic nerve activation evoked by stimulation of the sciatic nerve or nasal mucosa was pronounced in IH-exposed rats (179),

suggesting that IH sensitizes sympathetic nerve responses to other stimuli. IH affects the coupling of sympathetic-respiratory outputs at the CNS. The increased sympathetic nerve activity by IH coincides with the late expiratory phase of respiration (40, 204). A recent study by Moraes et al. (125) reported that a specific subpopulation of non-C1 respiratory-modulated rostral ventrolateral medullary (RVLM) presympathetic neurons exhibit enhanced excitatory synaptic inputs from the respiratory network, which may contribute to the increased sympathetic nerve activity in IH-exposed rats. Chemical sympathectomy with 6-OH dopamine prevents IH-induced hypertension (53), suggesting that enhanced sympathetic nerve activity contributes to elevated blood pressure.

Carotid Body Chemoreflex and Sympathetic Activation by IH

Carotid body being exquisitely sensitive to hypoxia is uniquely suited to sense and respond to even a modest drop in pO_2 that occurs during brief episodes of IH associated with recurrent apnea. Consequently, it was proposed that carotid bodies constitute the “frontline” defense system for detecting periodic hypoxemia associated with apneas and the resulting chemoreflex contributes to sympathetic nerve activation (22). The following section summarizes the role of carotid body chemoreflex in sympathetic activation by IH.

Clinical studies

Activation of the carotid body chemoreflex evokes sympatho-excitation in humans (29, 111, 177). The following lines of evidence suggest that the carotid body chemoreflex is augmented in recurrent apnea patients. First, hypoxia-induced sympathetic excitation, stimulation of breathing, and increase in blood pressure, all of which are hallmarks of the chemoreflex, are more pronounced in recurrent apnea patients than in control subjects (68, 91, 132). Second, in OSA patients, brief hyperoxic exposure, which decreases carotid body sensory activity, results in a more pronounced ventilatory depression (91, 189) and greater reduction in blood pressure (132) as compared with control subjects. Third, carotid body ablated subjects with sleep apneas do not develop hypertension [see “Discussion of the article” in reference (180)]. Collectively, these studies suggest that recurrent apnea patients exhibit heightened carotid body chemoreflex, which contributes to augmented sympathetic nerve activity.

Studies on animal models

Available evidence suggests that augmented carotid body chemoreflex also contributes to IH-induced sympathetic activation in experimental animals. IH-exposed cats (166) and mice (153) exhibit enhanced ventilatory response to hypoxia, a hallmark of carotid body chemoreflex. Huang et al. (79) reported exaggerated renal nerve responses to hypoxia and hypercapnia in rats exposed to 3 weeks of IH. Likewise, rats exposed to IH also exhibit enhanced thoracic sympathetic and phrenic nerve responses to cyanide, a potent stimulator of the carotid body in a working heart-brainstem preparation (12, 204).

Processing of sensory information from the carotid body at the CNS is critical for translating the increased carotid body activity to appropriate reflex activation of the sympathetic nervous system. The dorsal and medial subnuclei of the nucleus tractus solitarius (NTS),

including the commissural part of the NTS (cNTS), receive inputs from the CSN (20,201). Rats exposed to IH exhibit increased FosB/ FosB expression in the NTS and RVLM (97) as well as c-Fos protein expression in the dorsal and medial subnuclei of the NTS (178). These findings indicate that IH activates brainstem regions that regulate sympathetic preganglionic neuronal activity via the carotid body chemoreflex.

Neuronal activity in cNTS is regulated by various neurotransmitters, including glutamate (Glu), an excitatory amino acid transmitter, and dopamine (DA), an inhibitory biogenic amine. IH upregulates *N*-methyl-D-aspartate receptor 1 (NMDA-R1) expression in the dorsocaudal brainstem (164), Glu receptor types 2/3 subunit expression in cNTS (27), and AMPA- and NMDA-mediated currents in NTS neurons (33). On the other hand, IH downregulates tyrosine hydroxylase expression in the dorsal medulla, the rate-limiting enzyme in DA synthesis (59). Since DA inhibits synaptic transmission at the NTS (96), it is likely that IH, by downregulating the synthesis of DA, enhances glutamatergic excitatory transmission in the NTS (19). Kline et al. (95) showed that IH not only increases postsynaptic neuronal activity in the NTS but also attenuates synaptic transmission between sensory afferents and the NTS second-order neurons. This effect seems to occur via reduced transmitter release involving calcium/calmodulin-dependent kinase II activation (95).

Neurons in the NTS relay chemoafferent information to the hypothalamic paraventricular nucleus (PVN) and brain-stem sympathoexcitatory sites located in the RVLM (72,173). An earlier study by Greenberg et al. (61) showed that IH alters neuronal activity of the ventrolateral medulla (A1 noradrenergic cells). Zoccal et al. (203) reported that IH-induced sympathetic activation is mediated by enhanced purinergic but not glutamatergic transmission in the RVLM of juvenile rats. On the other hand, Silva and Schreihofer (179) found that glutamatergic transmission in the RVLM is critical for the augmented sympathetic activation by IH in adult rats. These findings implicate that age is an important variable that determines the type of neurotransmitter(s) mediating the effects of IH on sympathetic activation by RVLM. A recent study by Coleman et al. (24) reported that PVN neurons in IH-exposed mice exhibit decreased NMDA-R-mediated currents, reduced nitric oxide (NO) production by NMDA, and downregulation of NMDA-receptors in neuronal NO synthase-positive neurons. Collectively, these studies indicate that the exaggerated chemoreflex-mediated sympathetic activation by IH involves reconfiguration of neurotransmitter profiles in the CNS.

Fletcher and his co-workers reported that ablation of CSNs prevents IH-induced hypertension (52, 109). The CSN carries the sensory information from both baro-, and chemoreceptors. To delineate the contribution of chemoreceptors, Peng et al. (152) selectively ablated the carotid body using cryo-coagulation approach, while preserving the carotid baroreceptor function. Consistent with the earlier reports by Fletcher and his co-workers (52, 109), selective carotid body ablation prevented IH-induced hypertension and abolished elevated plasma catecholamine levels in IH-exposed rats (152).

OSA patients exhibit marked elevations in blood pressure during apneic episodes (81,186), which were attributed to the augmented sympathetic nerve activity affecting the vascular tone (92). To assess whether carotid body activation during apneic episodes contributes to

blood pressure elevations, Peng et al. (152) simulated apneas by exposing anesthetized rats to acute intermittent hypoxia (AIH; 12% O₂ for 15 s and then room air for 5 min, repeated for 10 cycles). Unlike the control rats, IH-exposed rats, similar to OSA patients, exhibited a marked elevation in blood pressures during each episode of AIH. Remarkably, selective ablation of the carotid bodies completely prevented the abnormal blood pressure increases seen with each episode of AIH (152). These findings suggest that carotid body activation during apneic episodes mediates the abnormal increases in blood pressures during AIH via carotid body chemoreflex.

Effects of IH on Carotid Body Function

Increased carotid body sensory nerve activity is a prerequisite for initiating the chemoreflex. Consequently, several studies examined the effects of IH on the carotid body sensory nerve activity in experimental animals and delineated the underlying cellular and molecular mechanisms. The following section summarizes the findings from these studies.

IH and carotid body sensory nerve response to hypoxia

Carotid body sensory nerve response to hypoxia was examined in rats that were exposed to IH for 10 days (148). Hypoxic sensory nerve response was augmented in IH-exposed rats as compared to those exposed to normoxia (Fig. 1). Similar effects of IH were also observed in cats (166) and mice (153), suggesting that IH uniformly augments the hypoxic sensory response in three species studied thus far. The enhanced hypoxic sensory response was completely reversed following reoxygenation of IH-exposed rats (147). In striking contrast, IH had no effect on carotid body response to hypercapnia (148) suggesting that IH leads to selective sensitization of the hypoxic response. Given that carotid chemoreflex is a potent activator of the sympathetic nervous system, it is likely that IH-induced augmented hypoxic sensitivity of the carotid body contributes to the pronounced increase in sympathetic nerve activity that occurs during each episode of IH.

Induction of sensory long-term facilitation of the carotid body by IH

In addition to the sensitization of the carotid body to hypoxia, IH induces a form of plasticity of the carotid body manifested as sensory long-term facilitation (LTF; 147). Acute IH (10 episodes of 15 s of 12% O₂ interspersed with 5 min of 95% O₂) progressively increased carotid body baseline sensory activity in IH-exposed rats, which persisted as long as an hour even after the termination of acute IH (Fig. 1). The long-lasting increase in baseline sensory activity was observed despite maintaining arterial blood gas composition and blood pressure comparable to control, preacute IH period (147). The time course of the carotid body sensory activity resembled that of LTF of breathing elicited by acute IH reported previously (124) and hence was referred to as sensory LTF. A notable difference between LTF of breathing and sensory LTF of the carotid body is that the former is seen in control naive rats in response to acute IH, wherein each episode of hypoxia lasts for 5 min. In contrast, sensory LTF of the carotid body is not seen in control, naive rats but requires prior exposure to IH for 10 days. However, a study by Cummings and Wilson (28) reported that acute intermittent hypocapnic hypoxia produces a weak sensory LTF in an *in vitro* perfused carotid body preparation from normoxia-exposed rats. Thus, these findings demonstrate that

IH induces a hitherto uncharacterized novel form of carotid body plasticity manifested as sensory LTF. It was proposed that the sensory LTF of the carotid body mediates the persistent elevation of the sympathetic nerve activity seen in IH-exposed rats (161). These studies establish that IH leads to remodeling of carotid body function manifested as sensitization of the hypoxic sensory response and induction of sensory LTF.

Factors affecting the carotid body response to IH

The effects of IH on the carotid body were seen only after three days of IH and the magnitude of the responses (i.e., sensitization of the hypoxic response and sensory LTF) further increased with ten days of IH exposure (147,148). Altering the severity of hypoxia used for IH conditions had little impact on the magnitude of the responses (147). These studies demonstrate that the effects of IH on the carotid body developed over time and were independent of the severity of hypoxia used for IH conditioning.

Carotid body responses to IH are unique

The total duration of hypoxia accumulated over ten days of IH equals to 4 h of continuous hypoxia. However, either a single episode of 4 h of hypoxia or 4 h of hypoxia per day for 10 days were ineffective in either evoking sensitization of the hypoxic response or eliciting the sensory LTF (147, 148). Thus, for a given duration of hypoxic exposure, IH is more effective than continuous hypoxia in altering the carotid body function. However, it has been reported that rats exposed to 3 days of continuous hypoxia also exhibit a sustained elevation of CSN activity following return to acute normoxia (18). A similar “after effect” of continuous hypoxia on sympathetic nerve activity and blood pressure was also reported in healthy humans who returned to sea level after 4 weeks of sojourn at high altitude (64).

Mechanisms Underlying the Effects of IH on the Carotid Body

Morphology of the carotid body

The carotid body is composed of two major cell types: the type I (also called glomus cells) and type II cells. A substantial body of evidence suggests that type I cells are the initial sites of hypoxic sensing and they work in concert with the nearby afferent nerve endings as a “sensory unit” (99). Chronic hypoxia results in hyperplasia of the glomus cells and hypertrophy as well as neo-vascularization of the carotid body (39, 66, 88, 102, 156). However, 10 days of IH exposure had no significant effect either on the number of glomus cells or total volume of the carotid body or glomus cell volume compared to controls (147). Del Rio et al. (38) also did not find any changes in the carotid body volume and the number of glomus cells in rats exposed to 21 days of IH. However, they found enlarged vascular area and increased size of the blood vessels, without any change in the number of the blood vessels in IH-exposed carotid bodies. The increased size of blood vessels was associated with upregulation of vascular endothelial growth factor (VEGF, 38). Collectively, these studies suggest that changes in the number of glomus cells or morphology of the carotid body do not account for the effects of IH on the carotid body function.

Ex vivo carotid bodies from IH-exposed animals still exhibited sensitization of the hypoxic sensory response as well as sensory LTF similar to those seen in *in vivo* preparations (147,

153), suggesting that the altered carotid body function is due to a direct effect of IH on glomus tissue rather than secondary to altered vascular perfusion caused by hypertension.

Glomus cell excitability and ion channels

Glomus cells express a variety of K⁺ channels including calcium-activated, human-ether-go-go (HERG)-like, and TASK-like K⁺ channels (13,99). Hypoxia by inhibiting one or more of these K⁺ channels depolarizes glomus cells leading to voltage-gated Ca²⁺ influx. The ensuing Ca²⁺-dependent release of excitatory neurotransmitter(s) stimulates the afferent nerve endings resulting in increased sensory discharge (99). Whether IH affects the excitability of glomus cells was examined.

Ortiz et al. (137) reported that IH had no effect on the resting membrane potential of glomus cells, whereas it increased the hypoxia-induced depolarization by two-fold. Moreover, the magnitude of hypoxia-evoked inhibition of TASK-like K⁺ channels was greater and the time course of the response was faster in IH-exposed glomus cells. It is likely that the enhanced inhibition of TASK-like K⁺ channels and the resulting greater depolarization contribute, in part, to the augmented hypoxic sensory response of the IH-exposed carotid body; whether IH also affects other K⁺ channels in glomus cells remains to be investigated.

Neurotransmitter(s) in IH-Evoked sensitization of the hypoxic sensory response

Carotid body synthesizes and stores a variety of neurotransmitters/modulators (159), which are shown to play important roles in the sensory nerve response to hypoxia (99). Since the effects of IH on the carotid body are reversible, it was proposed that IH recruits additional neurotransmitters or modulators in the carotid body, which otherwise, play a little or no role in the hypoxic sensory nerve response in control carotid bodies (160).

Endothelin-1 (ET-1)—Endothelin-1 (ET-1) is a vasoactive peptide. Biological actions of ET-1 are mediated via G-protein coupled receptors designated as ET_A, ET_B, and ET_C. Under basal conditions, ET-1 is expressed at very low levels in the carotid body and ET_A receptor antagonists had no effect on the hypoxic sensory response (17), suggesting that ET-1 has a limited or no role in normal carotid body function. However, ET-1 is a potent modulator of the hypoxic sensory response. Exogenous administration of ET-1 augments the hypoxic sensory response without altering the baseline activity in *ex vivo* carotid bodies, wherein vasoconstrictor effects of the peptide are effectively absent, indicating that ET-1 acts directly on the chemoreceptor tissue (17). An ET_A but not an ET_B receptor antagonist prevents the exogenous effects of ET-1 on the rat carotid body (17,18). Since plasma levels of ET-1 are elevated in rats exposed to IH (90) and in OSA patients (170), recent studies examined the role of ET-1 in IH-induced sensitization of the carotid body response to hypoxia.

Iturriaga and co-workers were one of the first to report that IH increases ET-1 expression in the carotid body and bosentan, a pan ET-1 receptor antagonist prevents IH-induced sensitization of the hypoxic sensory response (Fig. 2; 165–168). It was proposed that the transcriptional activator, hypoxia-inducible factor-1 (HIF-1) contributes to IH-induced upregulation of ET-1 in the carotid body (107). However, Peng et al. (145) found that IH

had no effect on pre-pro ET-1 mRNA expression in the carotid body, whereas elevated ET-1 levels were due to increased processing from its precursor, pre-pro endothelin involving activation of the endothelin-converting enzyme (ECE). They further showed that hypoxia facilitates ET-1 release from IH-treated but not from the control carotid body. In IH-exposed carotid bodies, mRNAs encoding ET_A receptor were upregulated and an ET_A receptor specific antagonist abolished IH-induced hypersensitivity of the carotid body to hypoxia (145). Exposing rats to more than one week of IH seems to reduce the intensity of ET-1-like immunoreactivity in the carotid body (38), indicating that ET-1 contributes to the sensitization of the hypoxic response at least in the initial stages of IH exposure.

Interestingly, IH upregulates ET-1 levels in the carotid sinus region of rats, which is the site of baroreceptor nerve endings. The increased ET-1 expression in the carotid sinus region was associated with increased ECE activity, which generates biologically active ET-1 (142). Carotid baroreceptor responses to elevated sinus pressures were attenuated in IH-exposed rats, and ET_A receptor antagonist prevented this response (142). A similar reduction in baroreflex-regulated sympathetic nerve activity was also reported in healthy humans exposed to 2 weeks of IH (191). These observations suggest that IH-induced increase in ET-1 on one hand, leads to the sensitization of the carotid body response to hypoxia, whereas it attenuates carotid baroreceptor activity. Since chemoreflex stimulates, whereas baroreflex inhibits sympathetic nerve activity, it was proposed that ET-1-mediated imbalance between carotid chemo-, and baro-reflex contributes to the enhanced sympathetic activation by IH (142).

Inflammatory cytokines—A recent study reported that IH-exposed carotid bodies exhibit inflammatory phenotype as evidenced by the increase in the number of ED1 positive macrophages (105). Immunocyto-chemical analysis revealed an increase in the expression of tumor necrosis factor- α , interleukin-1 β and accumulation of p65, a Nuclear Factor (NF)- κ b subunit in IH-exposed rat carotid bodies (36, 37). Although ibuprofen prevented IH-induced upregulation of proinflammatory cytokines, it had no effect on the hypersensitivity of the carotid body to hypoxia (36). Therefore, it is unlikely that the pro-inflammatory cytokines contribute to the IH-evoked heightened carotid body response to hypoxia.

Neurotransmitters mediating the sensory LTF

A subset of neurotransmitters/modulators distinct from those mediating the heightened hypoxic sensitivity seem to contribute to the induction of sensory LTF of the carotid body. Thus, ET_A receptor antagonist that prevented the heightened carotid body response to hypoxia, showed no effect on the sensory LTF (142). Furthermore, mimicking IH with exogenous spaced application of ET-1 was also found to be ineffective in inducing sensory LTF in the control carotid body (142). Likewise, spaced application of a number of putative excitatory neurochemicals including acetylcholine (ACh), adenosine triphosphate (ATP) and Substance P (SP) as well as KCl, a nonselective depolarizing agent although stimulated the carotid body, but were ineffective in evoking the sensory LTF (149). In striking contrast, spaced application of 5-hydroxytryptamine (5-HT) or angiotensin II (Ang II) produced a robust sensory LTF in control carotid bodies (149, 151), suggesting that they contribute to

IH-induced sensory LTF. The following section summarizes the studies examining the roles of 5-HT and Ang II in IH-induced sensory LTF.

5-hydroxytryptamine—It is known that 5-hydroxytryptamine (5-HT) evokes long-lasting neuronal activation in the nervous system (116, 121). Carotid body expresses substantial amounts of 5-HT (84). Peng et al. (146) examined the role of 5-HT in IH-induced sensory LTF. They found that hypoxia facilitates 5-HT release from IH but not from the control carotid bodies. The hypoxiaevoked 5-HT release in IH-exposed carotid body was prevented by 2-APB, a purported inositol phosphate-3 (IP3) receptor/TRPM2 channel blocker (157) but not by cadmium chloride, a broad-spectrum voltage-gated Ca^{2+} channel blocker (146). These findings suggest that IH evoked 5-HT release involves calcium signaling by IP3 rather than voltage-gated Ca^{2+} influx. Mice deficient in the gene encoding the Pet-1 transcription factor exhibit markedly reduced 5-HT expression in the CNS (70, 71). Pet-1^{-/-} mice carotid bodies also exhibited a similar absence of 5-HT-like immunoreactivity in glomus cells which is associated with a striking absence of IH-induced sensory LTF (146). These findings suggest that 5-HT mediates IH-induced sensory LTF.

Angiotensin II—Carotid bodies synthesize angiotensin II (Ang II) via a renin-independent pathway (103). Glomus cells express angiotensin-converting enzyme (ACE) (104, 112) and angiotensinogen, the precursor of Ang II, as well as angiotensin type 1 receptor (AT1R) (103, 112). Although the role of Ang II in “normal” carotid body function remains to be established, it has been suggested that Ang II mediates the exaggerated chemoreflex response to hypoxia in experimental models of congestive heart failure (113, 119). Spaced application of as little as picomolar concentration of Ang II induces robust sensory LTF in control carotid bodies, and this effect was prevented by losartan, an AT1R blocker (149). A recent study reported that IH upregulates angiotensinogen, ACE and AT1R in rat glomus cells (106). Taken together, these findings suggest that Ang II also potentially contribute to IH-induced sensory LTF, a possibility that requires further investigation. Since both 5-HT and Ang II produce sensory LTF of the carotid body, it is likely that an interplay between the signaling pathways initiated by these two neuromodulators contribute to IH-induced sensory LTF.

Cellular mechanisms: Reactive oxygen species

It was proposed that reactive oxygen species (ROS) generated during the reoxygenation phase contribute to carotid body responses to IH (160, 197). ROS levels were increased in IH-exposed carotid bodies as evidenced by decreased aconitase enzyme activity (147), an *in vivo*, robust biochemical marker of ROS (56), increased malondialdehyde levels, an index of oxidized lipids (145) and serum 8-isoprostane and nitrotyrosine, markers of oxidative stress (105). Moreover, treating IH-exposed rats with a stable, membrane permeable superoxide dismutase mimetic, [manganese(III)tetrakis(1-methyl-4-pyridyl)porphyrin pentachloride (MnTMPyP), 5 mg/kg, i.p.], a potent scavenger of ROS completely prevented the sensitization of the hypoxic response and the sensory LTF (147, 148). Similar effects of other antioxidants on IH-induced carotid body responses were also reported (35). A single application of MnTMPyP on the last day of IH treatment, instead of daily administration prior to IH, failed to prevent the effects of IH on the carotid body (147). These findings

suggest that ROS-mediated signaling cascade rather than ROS generation *per se* is critical for carotid body responses to IH. Interestingly, OSA patients also exhibit increased ROS levels as compared with control subjects (21, 188).

Sources of ROS

Mitochondrial electron-transport chain—One of the cellular sources of ROS generation involve inhibition of complexes I and III of the mitochondrial electron-transport chain (ETC; 2). Biochemical analysis revealed markedly reduced complex I but not the complex III activity in IH-exposed carotid bodies (147). The decreased complex I activity was associated with increased mitochondrial ROS (93, 197). Thus, these observations suggest that inhibition of mitochondrial ETC at complex I is an important source of ROS in IH-exposed carotid bodies. Unlike IH, continuous hypoxia inhibits complex III (62), highlighting important differences in mitochondrial sources of ROS generation between both forms of hypoxia.

NADPH oxidases—In addition to the mitochondrial ETC, cytosolic and membrane-bound oxidases constitute important additional sources of ROS (63). Among them, the Nox family of enzymes including Nox1, 2, 3, and 4 isoforms are major sources of ROS (9). Carotid bodies express mRNAs encoding all four isoforms of Nox. IH increases Nox2 mRNA levels in the carotid bodies by 35-fold; whereas mRNA levels of Nox1 and Nox3 increased by 16- and 13-fold, respectively (146). A similar IH-induced increase in Nox2 in the carotid body was also reported by Lam *et al.* (105). Nox1 is a homolog of Nox2 (5, 187).

Immunocytochemical analysis revealed that Nox2 and Nox4 protein levels are increased in the cytosol and nuclei, respectively of glomus cells of IH-exposed carotid bodies (146). Losartan, an AT1R blocker prevented IH-induced upregulation of Nox2 mRNA (106). IH-induced sensory LTF was absent in mice deficient in gp91^{phox}, the catalytic subunit of the Nox2 complex as well as following pharmacological blockade of Nox by apocynin or by AEBSF (146). These observations suggest that IH leads to transcriptional upregulation of Nox2 in the carotid body, which contributes to the induction of sensory LTF. The role of Nox4, which is also upregulated by IH, however, remains to be examined.

Evidence for ROS-induced ROS—Recently, Khan *et al.* (93) investigated potential interactions between Nox2 and the mitochondrial complex I. The following findings of this study suggest that ROS generated by Nox2 mediate complex I inhibition by IH: (i) Nox inhibitors or loss of Nox2 function by silencing RNA approach prevent complex I inhibition by IH and (ii) complex I inhibition was absent in IH treated gp91^{phox} knockout mice. This study further showed that ROS generated by Nox2 facilitates Ca²⁺ influx into mitochondria resulting in oxidative modification of the complex I as evidenced by S-glutathionylation of 75- and 50-kDa subunits, which results in inhibition of the complex I activity. After terminating IH, Nox2-mediated ROS generation returned to control levels within 3 h, whereas complex I-mediated ROS generation persisted as long as 16 h (93). These findings suggest that there is a cross-talk between the membrane-bound Nox2 and the mitochondrial complex I leading to ROS-induced ROS (positive “feed-forward” mechanism) resulting in long-lasting ROS generation by IH.

Other oxidases—Xanthine oxidoreductase (XO) system is another major source of cellular ROS (122, 123, 134). It is comprised of xanthine dehydrogenase and XO, which catalyze the oxidation of purines generating superoxide radical as a by product. A recent study reported that XO activation by IH contributes, in part, to increased ROS (128). Whether XO is also activated in the carotid body by IH, however, has not been examined.

Antioxidant enzymes—Cellular ROS levels depend on the balance between their generation by pro-oxidant enzymes and degradation by antioxidant enzymes. A recent study showed that IH downregulates the *Sod2* gene, which encodes superoxide dismutase 2 (Sod2), a major antioxidant enzyme (129). IH decreases Sod2 enzyme activity in the carotid bodies (129). These findings suggest that IH-induced oxidative stress in the carotid body is due to increased ROS generation resulting from inhibition of the mitochondrial ETC at complex I, as well as transcriptional upregulation of pro-oxidants like Nox2, with a concomitant downregulation of antioxidant enzymes such as Sod-2.

Molecular mechanisms underlying ROS generation by IH

As mentioned above, the effects of IH on the carotid body develop over time. Generally, the time-dependent changes in the physiological responses are attributed to transcription factor-mediated gene regulation and the resulting protein products (163). IH activates a variety of transcription factors, including the HIFs, c-Fos (a component of the activator protein-1 complex, AP1), nuclear factor of activated T-cells (NFAT), and NF- κ B (130). Recent studies demonstrated that transcriptional regulation by HIFs play a critical role in IH-induced ROS generation in the carotid body by affecting the pro-, and anti-oxidant enzyme gene expression.

Hypoxia-inducible factor-1—The transcriptional activator, hypoxia-inducible factor-1 (HIF-1) is a master regulator of O₂ homeostasis during hypoxia that controls multiple physiological processes and it regulates the expression of hundreds of genes (118). HIF-1 comprises an O₂-regulated subunit and a constitutive β -subunit (174). Recent studies showed that IH is a potent activator of HIF-1 in cell cultures (199, 200), and in the CNS of mice (153). Although Lam et al. (108) reported that IH increases HIF-1 α transcription but not the protein, subsequent studies showed that IH increases HIF-1 α protein involving decreased protein degradation by proline hydroxylation, and increased protein synthesis by the mammalian target of rapamycin (25, 199).

Complete HIF-1 α deficiency results in embryonic lethality at mid-gestation, whereas *hif1a*^{+/-} heterozygous (HET) mice, which are partially deficient in HIF-1 α expression, develop normally and are indistinguishable from wild-type (WT) littermates under normoxic conditions (83,196). Recent studies examined whether HIF-1 contributes to carotid body responses to IH (153). Carotid bodies of IH-exposed *hif1a*^{+/-} mice exhibit remarkable absence of sensitization of the hypoxic sensory response, sensory LTF and unaltered ROS levels; whereas these phenotypic responses were seen in WT mice treated with IH (153). A recent study reported that HIF-1 mediates transcriptional activation of Nox2 (198). Therefore, it is likely that activation of HIF-1 by IH contributes to carotid body response to

IH via transcriptional upregulation of Nox2, a major pro-oxidant enzyme and the resultant increase in ROS.

Hypoxia-inducible factor-2—Hypoxia-inducible factor-1 (HIF-2 α) (also known as endothelial PAS domain protein-1, EPAS-1) is another member of the HIF family (193). HIF-2 α shares ~50% sequence homology to HIF-1 α and also interacts with HIF-1 β (45). Continuous hypoxia activates both HIF-1- and HIF-2-mediated transcriptions, which in turn regulate several common target genes (77). Nanduri et al. (129) reported that unlike continuous hypoxia, IH downregulates HIF-2 α protein in the carotid body and this effect is mediated by Ca²⁺-dependent protease, calpain. These authors further showed that IH-induced HIF-2 α degradation results in transcriptional downregulation of mRNAs encoding several antioxidant enzymes including the Sod-2 in the carotid bodies (144). Remarkably, *hif2a*^{+/-} mice exhibit several phenotypic responses that are similar to IH treated WT mice, including augmented carotid body response to hypoxia, elevated blood pressures, increased plasma catecholamines, disrupted breathing manifested by apneas, and oxidative stress (144). Antioxidant treatment normalizes carotid body function, and restores cardio-respiratory functions of *hif2a*^{+/-} mice. These observations suggest that down-regulation of HIF-2 α contributes to IH-induced elevation of ROS via decreased transcription of antioxidant enzymes.

Thus, the imbalance between HIF-1 and HIF-2 emerges as a major molecular mechanism underlying IH-induced sensitization of the hypoxic response and sensory LTF of the carotid body. Furthermore, HIF- α isoforms also regulate genes encoding various ion channels (163, 171) as well as enzymes involved in the synthesis of transmitters including ACE (163). Further studies are needed to identify other HIF-dependent target genes in addition to those associated with redox regulation that may also potentially contribute to altered carotid body function by IH. The cellular and molecular mechanisms underlying the effects of IH on the carotid body and the resultant chemoreflex-mediated changes in blood pressure are schematically illustrated in Figure 3.

How do ROS contribute to IH-induced altered carotid body function?

Recent studies reported that IH-induced increase in ET-1 in the carotid body, which mediates the sensitization of the hypoxic response requires ROS-dependent activation of ECE (142, 145). In addition, ROS also contributes to hypoxia-induced ET-1 release from IH-exposed carotid bodies (145). Nox2-derived ROS is also required for the induction of sensory LTF. Nox2 activation in IH-exposed carotid bodies require activation of protein kinase C either by 5-HT₂ receptors (146) and/or AT₁R (149). Since IH enhances depolarization of glomus cells involving inhibition of TASK-like K⁺ channels (137), it remains to be determined whether ROS also play a role in the enhanced glomus cell excitability by IH.

The family of ROS includes superoxide anion (O₂^{•-}), and its dismutated product hydrogen peroxide (H₂O₂) as well as hydroxyl radical (OH[•]). A study by Peng et al. (146) provided important insights into the chemical identity of ROS mediating the effects of IH on the carotid body. Priming the control carotid body with nanomolar concentrations of H₂O₂

alone is sufficient to evoke both the augmented hypoxic sensory response as well as the induction of sensory LTF by acute IH similar to those observed in IH treated carotid bodies. Treating rats with MnTMPyP, an $O_2^{\cdot-}$ scavenger, prior to IH completely prevents sensitization of the hypoxic response as well the induction of sensory LTF (147, 148). However, after the induction of sensory LTF, application of MnTMPyP was ineffective in preventing the maintenance phase of the sensory LTF; whereas, catalase, which degrades H_2O_2 , effectively blocked the maintenance of sensory LTF in IH treated carotid bodies (146). These findings taken together suggest that IH-induced remodeling of the carotid body function requires both $O_2^{\cdot-}$ and its stable dismutated product H_2O_2 .

IH-induced HIF imbalance and oxidative stress in end organs require sympathetic activation by carotid body chemoreflex

Adrenal medulla is a major end-organ of the sympathetic nervous system. In adult rats, adrenal chromaffin cells are relatively insensitive to the direct effect of hypoxia (192). However, in IH-exposed rats, hypoxia releases catecholamines from the adrenal medulla by directly acting on chromaffin cells (98). This IH-induced hypoxic sensitivity of the adrenal chromaffin cells was shown to be due to increased ROS levels (98). Originally the increased ROS levels were attributed to the direct effect of IH on the adrenal medullary chromaffin cells (AMCs). The IH paradigm employed in this study (98) produced only a modest decrease in arterial blood O_2 saturation (~from 97% to 80% or ~20–25 mmHg). Under normoxia although arterial blood pO_2 is ~100 mmHg, pO_2 levels of most tissues including adrenal medulla are much lower and range anywhere between 30 and 60 mmHg (15). Given these low tissue pO_2 levels, modest fluctuations in arterial pO_2 caused by IH may not be sufficient to induce oxidative stress in the adrenal medulla. Peng et al. (152) examined whether carotid body chemoreflex mediates oxidative stress in IH-exposed adrenal medulla. Their results showed that either selective ablation of the carotid body (i.e., removal of the afferent pathway) or chronic ablation of sympathetic innervation to the adrenal medulla (i.e., removal of the efferent pathway) prevented the induction of oxidative stress in the adrenal medulla by IH. These findings suggest that sympathetic activation by the carotid body chemoreflex rather than the direct effect of IH mediates oxidative stress in the adrenal medulla. Analysis of molecular mechanisms revealed that the increased oxidative stress in the adrenal medulla is due to muscarinic acetylcholine receptor-mediated imbalance between HIF- α isoforms resulting in transcriptional dysregulation of pro-, and anti-oxidant enzyme genes. Thus, this study (152) unraveled a hitherto uncharacterized carotid body chemoreflex-dependent regulation of the redox state and HIF-mediated transcriptional regulation in the end-organ such as the adrenal medulla. Moreover, either adrenal demedullation (6) or ablation of the carotid body or sympathetic innervation to the adrenal medulla (152) prevented IH-induced hypertension in rats. These studies suggest that in addition to controlling the vascular tone, chemoreflex mediated sympathetic activation also contributes to IH-induced hypertension by enhancing catecholamine secretion from the adrenal medulla. Whether sympathetic activation by IH also leads to oxidative stress in other end-organs such as blood vessels remains to be studied.

Is the Carotid Body A Therapeutic Target of IH-Induced Hypertension?

As described above, currently available CPAP therapy is ineffective in normalizing blood pressures in a subset of OSA patients with resistant hypertension (43). Consequently, there is an unmet clinical need for alternative therapeutic strategies for treating hypertension associated with IH resulting from SDB with apnea. Although selective ablation of the carotid body prevented hypertension in a rodent model of IH (52, 109, 152), use of carotid body ablation as a therapeutic intervention in humans has serious limitations. Carotid body reflexes are critical for a number of physiological functions including high altitude adaptation, maintenance of blood gases during exercise, and cardio-respiratory responses to short-term hypoxia (99). Consequently, all of these functions will be adversely affected by carotid body ablation. Alternatively, reducing the carotid body hypersensitivity by pharmacological means has the potential to normalize blood pressure in recurrent apnea patients. Since in experimental animal models either antioxidants or blockers of ET_A, 5-HT₂ and AT₁ receptors prevented IH-induced hypersensitivity of the carotid body as well as the sensory LTF (145–148, 151), they either alone or in combination with CPAP, could be used as potential therapeutic interventions in controlling blood pressures in recurrent apnea patients. Supporting such a possibility, a recent study reported that valsartan, an antihypertensive medication produced a four-fold higher decrease in mean 24-h blood pressure than CPAP alone in untreated hypertensive patients with OSA (154). Moreover, recent studies showed that hydrogen sulfide (H₂S) generated by the enzyme cystathionine- γ -lyase (CSE) is a critical mediator of hypoxic sensing by the carotid body (117, 141). Interestingly, treating spontaneous hypertensive rats with L-propargylglycine, an inhibitor of CSE, was found to be as effective as carotid body ablation in reducing blood pressures (143). These observations provide alternative strategy for assessing the efficacy of CSE inhibitor(s) as another potential pharmacological intervention for preventing hypersensitivity of the carotid body and normalizing blood pressures in a subset of recurrent apnea patients.

IH in Neonates

Recurrent apnea is a major clinical problem in infants born preterm (apnea of prematurity). Depending on the gestational age, recurrent apneas are prevalent in nearly 90% of the preterm infants (1, 158). Infants with recurrent apnea exhibit autonomic dysfunction with clinical signs of over-active sympathetic nervous system (100), altered sympathoadrenal function (101), augmented ventilatory response to hypoxia (135), and cardiac arrhythmias (158).

Carotid body responses to neonatal IH and the underlying mechanisms

Unlike adults, in neonates, hypoxia is not sensed by the carotid bodies because they are developmentally immature (11, 16, 42, 57). In rats, maturity of the carotid body develops during the first two weeks of life (16, 42, 94). Perturbations in environmental O₂ in the neonatal period profoundly impact the developmental maturation of the carotid bodies (16,42,65). For instance, exposure of rat pups to either chronic hypoxia (16,42,65,185) or to hyperoxia (10) delays the developmental maturation of the carotid body. On the other hand, carotid bodies from neonatal rat pups exposed to IH exhibit markedly augmented hypoxic sensory response, similar to that seen in adult rats (140, 150; Fig. 4). This IH-induced

sensitization of the hypoxic sensory response in neonatal rat pups was also shown to be mediated by ROS signaling (139).

A study by Pawar et al. (140) delineated the following striking differences in the carotid body responses to IH between adult and neonatal rats. First, the magnitude of IH-evoked hypoxic sensitization was significantly greater in neonates than in adults. Second, as short as 8 h of IH was sufficient to evoke sensitization of the hypoxic response in neonatal rat pups as opposed to the requirement of 3 days of IH to elicit a similar response in adults. Third, unlike in adults, IH did not induce sensory LTF in neonatal carotid bodies. Fourth, IH led to hyperplasia of glomus cells in neonates, whereas it had no effect on the number of glomus cells in adult carotid bodies. Fifth, IH-induced hypoxic sensitization and oxidative stress were reversed in adult rats after reoxygenation for 10 days, whereas those induced by neonatal IH persisted into adult life (2 months old).

Endothelin-1 (ET-1)—Since endothelin-1 (ET-1) signaling plays an important role in IH-induced sensitization of the hypoxic response in the adult carotid body (145,165,167,168), Pawar et al. (139) examined whether ET-1 also contributes to the heightened hypoxic sensitivity in neonates. Their results showed that: (i) unlike the adult carotid body, neonatal chemoreceptor tissue expresses high basal levels of ET-1 in glomus cells, (ii) IH exposure markedly facilitated basal ET-1 release from neonatal carotid bodies, (iii) ET_A but not ET_B receptor antagonist prevented IH-induced sensitization of the hypoxic response, and (iv) antioxidant treatment prevented the effects of neonatal IH. Thus, these studies indicate that ET-1 is a major mediator of the effects of neonatal IH on the carotid body.

Other mechanisms—The effects of IH on neonatal glomus cells have not yet been investigated. On the other hand, mechanism(s) underlying the cellular effects of neonatal IH had been investigated in neonatal AMCs, which like carotid body glomus cells are also exquisitely sensitive to hypoxia (190,192). Souvannakitti et al. (183,184) reported that IH markedly augments hypoxia-induced exocytosis from neonatal AMC, which is in part due to ROS-induced upregulation of Ca_v3.2 T-type Ca²⁺ channels and increased mobilization of intracellular Ca²⁺ stores from ryanodine receptors, especially of the RyR2 subtype. Given the similarities between neonatal AMC and carotid body glomus cells, it is likely that similar Ca²⁺ signaling mechanisms might also contribute to neonatal IH-induced augmented carotid body hypoxic sensitivity, a possibility that requires further investigation.

Consequences of neonatal IH-induced carotid body hypersensitivity in adult life

As described above, neonatal IH-induced hypersensitivity of the carotid body persisted in adult rats (140). This effect was associated with elevated plasma catecholamines, hypertension, and irregular breathing with apneas (87,127). Therefore, it is conceivable that the augmented chemoreflex contribute to hypertension and respiratory abnormalities in adult rats that were exposed to IH in the neonatal period. Recent clinical studies reported a higher incidence of SDB (75, 138, 169), hypertension, and insulin resistance (31) in adults who were born preterm than those born full-term. Collectively, the above results suggest that the long-lasting effects of neonatal IH predispose to early onset of cardio-respiratory diseases in adult life.

Epigenetic Programming by Neonatal IH

What mechanisms mediate the long-lasting effects of neonatal IH? Environmental factors during prenatal and early postnatal periods influence developmental programming of homeostatic mechanisms (7, 23, 58). Emerging evidence suggests that aberrant epigenetic regulation is an underlying mechanism for producing long-lasting effects of environmental perturbations in neonatal life. Epigenetic changes are heritable modifications of DNA including DNA methylation and histone modifications (47, 85). DNA methylation occurs predominantly on cytosine bases that are part of a 5'-CpG-3' dinucleotide, catalyzed by three DNA methyltransferase enzymes (Dnmts) using S-adenosylmethionine as the methyl donor (46). DNA hypermethylation leads to repression of gene transcription whereas hypomethylation causes transcriptional activation (176).

Neonatal IH leads to DNA hypermethylation of antioxidant enzyme genes

A recent study examined the potential role of epigenetic mechanisms involving DNA methylation in the long-lasting effects of neonatal IH on the carotid body (127). This study reported that the augmented hypoxic sensitivity of the carotid body in adult rats exposed to neonatal IH was associated with persistent oxidative stress, decreased expression of genes encoding antioxidant enzymes, and increased expression of pro-oxidant enzymes. The decreased expression of the *Sod2* gene, which encodes the antioxidant enzyme, superoxide dismutase 2 was associated with DNA hypermethylation of a single CpG dinucleotide close to the transcription start site. IH increased the expression of Dnmt 3b, which catalyzes DNA hypermethylation. Treating neonatal rats with decitabine, an inhibitor of DNA methylation, during IH exposure prevented oxidative stress, the enhanced hypoxic sensitivity of the carotid body, and autonomic dysfunction in adult rats. The mechanism(s) contributing to the increased pro-oxidant enzymes, however, remains to be elucidated.

Altered programming of homeostatic mechanisms via epigenetic modulation during perinatal development has been proposed to be the cause of susceptibility to diseases in adulthood (3, 7, 41, 47, 76, 172). Consistent with this possibility, the above-described findings from the study by Nanduri et al. (127) implicate a hitherto uncharacterized role for epigenetic mechanisms in mediating the long-lasting effects of neonatal IH on the carotid body and the ensuing chemoreflex in initiating the early onset of cardio-respiratory dysfunction in adulthood (Fig. 5).

Gaps in the knowledge and future directions—Recurrent apnea with IH is a major clinical problem in adults and in preterm infants. Augmented sympathetic nerve activity and hypertension are established comorbidities associated with IH. In this review, we attempted to summarize the impact of IH on hypoxic sensing by the carotid body and the contribution of chemoreflex to sympathetic activation and hypertension. Studies on experimental animal models firmly established that IH profoundly affects the carotid body function which manifested as heightened response to hypoxia in adults and neonates, and sensory LTF in adults. Emerging evidence suggests that dysregulation of transcriptional activators, HIF-1 and HIF-2 and the resulting imbalance in pro-, and anti-oxidant enzyme genes leading to elevated ROS signaling as the major molecular and cellular mechanism, respectively that

mediates the effects of IH on the carotid body. Although available evidence suggest that ROS signaling impacts neurotransmitter signaling in the carotid body, little is known on the effects of ROS on glomus cell excitability which is critical for carotid body sensory transduction (99). Although in adults the effects of short-term IH are reversible, the consequences of long-term IH are yet to be delineated. While a recent study (127) implicates epigenetic mechanisms involving DNA hypermethylation for mediating the long-term effects of neonatal IH, whether histone modifications which represent another important epigenetic mechanism also play a role remain an important area of future research. Future development of new therapeutic interventions targeting the carotid body hypersensitivity to hypoxia has considerable translational potential in controlling resistant hypertension in a subset of OSA patients, and early onset of cardiovascular diseases in adults born preterm.

Acknowledgements

Research from author's laboratories is supported by the National Institutes of Health Grant PO1-HL-90554 and UH2-HL-123610.

References

1. Abu-Shaweesh JM, Martin RJ. Neonatal apnea: What's new? *Pediatr Pulmonol*. 2008; 43:937–944. [PubMed: 18780339]
2. Ambrosio G, Zweier JL, Duilio C, Kuppusamy P, Santoro G, Elia PP, Tritto I, Cirillo P, Condorelli M, Chiariello M. Evidence that mitochondrial respiration is a source of potentially toxic oxygen free radicals in intact rabbit hearts subjected to ischemia and reflow. *J Biol Chem*. 1993; 268:18532–18541. [PubMed: 8395507]
3. Anway MD, Cupp AS, Uzumcu M, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science*. 2005; 308:1466–1469. [PubMed: 15933200]
4. Atkeson A, Yeh SY, Malhotra A, Jelic S. Endothelial function in obstructive sleep apnea. *Prog Cardiovasc Dis*. 2009; 51:351–362. [PubMed: 19249441]
5. Banfi B, Maturana A, Jaconi S, Arnaudeau S, Laforge T, Sinha B, Ligeti E, Demareux N, Krause KH. A mammalian H⁺ channel generated through alternative splicing of the NADPH oxidase homolog NOX-1. *Science*. 2000; 287:138–142. [PubMed: 10615049]
6. Bao G, Metreveli N, Li R, Taylor A, Fletcher EC. Blood pressure response to chronic episodic hypoxia: Role of the sympathetic nervous system. *J Appl Physiol*. 1997; 83:95–101. [PubMed: 9216950]
7. Barker DJ, Osmond C, Simmonds SJ, Wield GA. The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. *BMJ*. 1993; 306:422–426. [PubMed: 8461722]
8. Becker HF, Jerrentrup A, Ploch T, Grote L, Penzel T, Sullivan CE, Peter JH. Effect of nasal continuous positive airway pressure treatment on blood pressure in patients with obstructive sleep apnea. *Circulation*. 2003; 107:68–73. [PubMed: 12515745]
9. Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: Physiology and pathophysiology. *Physiol Rev*. 2007; 87:245–313. [PubMed: 17237347]
10. Bisgard GE, Olson EB Jr, Wang ZY, Bavis RW, Fuller DD, Mitchell GS. Adult carotid chemoafferent responses to hypoxia after 1, 2, and 4 wk of postnatal hyperoxia. *J Appl Physiol* (1985). 2003; 95:946–952. [PubMed: 12909596]
11. Blanco CE, Dawes GS, Hanson MA, McCooke HB. The response to hypoxia of arterial chemoreceptors in fetal sheep and new-born lambs. *J Physiol*. 1984; 351:25–37. [PubMed: 6747866]
12. Braga VA, Soriano RN, Machado BH. Sympathoexcitatory response to peripheral chemoreflex activation is enhanced in juvenile rats exposed to chronic intermittent hypoxia. *Exp Physiol*. 2006; 91:1025–1031. [PubMed: 16959820]

13. Buckler KJ. Two-pore domain k(+) channels and their role in chemoreception. *Adv Exp Med Biol.* 2010; 661:15–30. [PubMed: 20204721]
14. Carlson JT, Hedner J, Elam M, Ejnell H, Sellgren J, Wallin BG. Augmented resting sympathetic activity in awake patients with obstructive sleep apnea. *Chest.* 1993; 103:1763–1768. [PubMed: 8404098]
15. Carreau A, El Hafny-Rahbi B, Matejuk A, Grillon C, Kieda C. Why is the partial oxygen pressure of human tissues a crucial parameter? Small molecules and hypoxia. *J Cell Mol Med.* 2011; 15:1239–1253. [PubMed: 21251211]
16. Carroll JL. Developmental plasticity in respiratory control. *J Appl Physiol.* 2003; 94:375–389. [PubMed: 12486025]
17. Chen J, He L, Dinger B, Fidone S. Cellular mechanisms involved in rabbit carotid body excitation elicited by endothelin peptides. *Respir Physiol.* 2000; 121:13–23. [PubMed: 10854619]
18. Chen J, He L, Dinger B, Stensaas L, Fidone S. Role of endothelin and endothelin A-type receptor in adaptation of the carotid body to chronic hypoxia. *Am J Physiol Lung Cell Mol Physiol.* 2002; 282:L1314–L1323. [PubMed: 12003788]
19. Chen X, Kombian SB, Zidichouski JA, Pittman QJ. Dopamine depresses glutamatergic synaptic transmission in the rat parabrachial nucleus in vitro. *Neuroscience.* 1999; 90:457–468. [PubMed: 10215151]
20. Chitravanshi VC, Sapru HN. Chemoreceptor-sensitive neurons in commissural subnucleus of nucleus tractus solitarius of the rat. *Am J Physiol.* 1995; 268:R851–R858. [PubMed: 7733393]
21. Christou K, Markoulis N, Moulas AN, Pastaka C, Gourgoulialis KI. Reactive oxygen metabolites (ROMs) as an index of oxidative stress in obstructive sleep apnea patients. *Sleep Breath.* 2003; 7:105–110. [PubMed: 14569521]
22. Cistulli, PA.; Sullivan, CE. Pathophysiology of sleep apnea. In: Saunders, NA.; Sullivan, CE., editors. *Sleep and Breathing.* New York: Dekker; 1994. p. 405-448.
23. Cohen G, Jeffery H, Lagercrantz H, Katz-Salamon M. Long-term reprogramming of cardiovascular function in infants of active smokers. *Hypertension.* 2010; 55:722–728. [PubMed: 20100999]
24. Coleman CG, Wang G, Park L, Anrather J, Delagrammatikas GJ, Chan J, Zhou J, Iadecola C, Pickel VM. Chronic intermittent hypoxia induces NMDA receptor-dependent plasticity and suppresses nitric oxide signaling in the mouse hypothalamic paraventricular nucleus. *J Neurosci.* 2010; 30:12103–12112. [PubMed: 20826673]
25. Coleman ML, Ratcliffe PJ. Oxygen sensing and hypoxia-induced responses. *Essays Biochem.* 2007; 43:1–15. [PubMed: 17705789]
26. Comroe JH. The location and function of the chemoreceptors of the aorta. *Am J Physiol.* 1939; 127:176–191.
27. Costa-Silva JH, Zoccal DB, Machado BH. Chronic intermittent hypoxia alters glutamatergic control of sympathetic and respiratory activities in the commissural NTS of rats. *Am J Physiol Regul Integr Comp Physiol.* 2012; 302:R785–R793. [PubMed: 22204959]
28. Cummings KJ, Wilson RJ. Time-dependent modulation of carotid body afferent activity during and after intermittent hypoxia. *Am J Physiol Regul Integr Comp Physiol.* 2005; 288:R1571–R1580. [PubMed: 15677524]
29. Cutler MJ, Swift NM, Keller DM, Wasmund WL, Burk JR, Smith ML. Periods of intermittent hypoxic apnea can alter chemoreflex control of sympathetic nerve activity in humans. *Am J Physiol Heart Circ Physiol.* 2004; 287:H2054–H2060. [PubMed: 15242836]
30. Cutler MJ, Swift NM, Keller DM, Wasmund WL, Smith ML. Hypoxia-mediated prolonged elevation of sympathetic nerve activity after periods of intermittent hypoxic apnea. *J Appl Physiol.* 2004; 96:754–761. [PubMed: 14555683]
31. Dalziel SR, Parag V, Rodgers A, Harding JE. Cardiovascular risk factors at age 30 following pre-term birth. *Int J Epidemiol.* 2007; 36:907–915. [PubMed: 17468503]
32. De Castro F. Sur la structure et l'innervation de la glande intercarotidienne (glomus caroticum) de l'homme et des mammifères et sur un nouveau système de l'innervation autonome du nerf glossopharyngien. *Trav Lab Rech Biol.* 1926; 24:365–432.

33. de Paula PM, Tolstykh G, Mifflin S. Chronic intermittent hypoxia alters NMDA and AMPA-evoked currents in NTS neurons receiving carotid body chemoreceptor inputs. *Am J Physiol Regul Integr Comp Physiol.* 2007; 292:R2259–R2265. [PubMed: 17332161]
34. Deane BM, Howe A, Morgan M. Abdominal vagal paraganglia: Distribution and comparison with carotid body, in the rat. *Acta Anat (Basel).* 1975; 93:19–28. [PubMed: 1189897]
35. Del Rio R, Moya EA, Iturriaga R. Carotid body and cardiorespiratory alterations in intermittent hypoxia: The oxidative link. *Eur Respir J.* 2010; 36:143–150. [PubMed: 19996187]
36. Del Rio R, Moya EA, Iturriaga R. Contribution of inflammation on carotid body chemosensory potentiation induced by intermittent hypoxia. *Adv Exp Med Biol.* 2012; 758:199–205. [PubMed: 23080163]
37. Del Rio R, Moya EA, Parga MJ, Madrid C, Iturriaga R. Carotid body inflammation and cardiorespiratory alterations in intermittent hypoxia. *Eur Respir J.* 2012; 39:1492–1500. [PubMed: 22183481]
38. Del Rio R, Munoz C, Arias P, Court FA, Moya EA, Iturriaga R. Chronic intermittent hypoxia-induced vascular enlargement and VEGF upregulation in the rat carotid body is not prevented by antioxidant treatment. *Am J Physiol Lung Cell Mol Physiol.* 2011; 301:L702–L711. [PubMed: 21821731]
39. Dhillon DP, Barer GR, Walsh M. The enlarged carotid body of the chronically hypoxic and chronically hypoxic and hypercapnic rat: A morphometric analysis. *Q J Exp Physiol.* 1984; 69:301–317. [PubMed: 6729019]
40. Dick TE, Hsieh YH, Wang N, Prabhakar N. Acute intermittent hypoxia increases both phrenic and sympathetic nerve activities in the rat. *Exp Physiol.* 2007; 92:87–97. [PubMed: 17138622]
41. Dolinoy DC, Weidman JR, Jirtle RL. Epigenetic gene regulation: Linking early developmental environment to adult disease. *Reprod Toxicol.* 2007; 23:297–307. [PubMed: 17046196]
42. Donnelly DF. Developmental aspects of oxygen sensing by the carotid body. *J Appl Physiol.* 2000; 88:2296–2301. [PubMed: 10846048]
43. Dudenbostel T, Calhoun DA. Resistant hypertension, obstructive sleep apnoea and aldosterone. *J Hum Hypertens.* 2012; 26:281–287. [PubMed: 21654850]
44. Easton J, Howe A. The distribution of thoracic glomus tissue (aortic bodies) in the rat. *Cell Tissue Res.* 1983; 232:349–356. [PubMed: 6883447]
45. Ema M, Taya S, Yokotani N, Sogawa K, Matsuda Y, Fujii-Kuriyama Y. A novel bHLH-PAS factor with close sequence similarity to hypoxia-inducible factor 1 α regulates the VEGF expression and is potentially involved in lung and vascular development. *Proc Natl Acad Sci U S A.* 1997; 94:4273–4278. [PubMed: 9113979]
46. Fang JY, Xiao SD. Folic acid, polymorphism of methyl-group metabolism genes, and DNA methylation in relation to GI carcinogenesis. *J Gastroenterol.* 2003; 38:821–829. [PubMed: 14564626]
47. Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. *Nature.* 2007; 447:433–440. [PubMed: 17522677]
48. Fidone, SJ.; Gonzalez, C. Initiation and control of chemoreceptor activity in the carotid body. In: Fishman, AP.; Cherniack, NS.; Widdicombe, JG.; Geiger, SR., editors. *Handbook of Physiology- The Respiratory System- Control of Breathing.* Bethesda, MD, USA: American Physiological Society; 1986. p. 247-312.
49. Fitzgerald, RS.; Lahiri, S. Reflex responses to carotid/aortic body stimulation. In: Fishman, AP.; Cherniack, NS.; Widdicombe, JG.; Geiger, SR., editors. *Handbook of Physiology The Respiratory System Control of Breathing.* Bethesda, MD: American Physiological Society; 1986. p. 313-362.
50. Fletcher EC. An animal model of the relationship between systemic hypertension and repetitive episodic hypoxia as seen in sleep apnoea. *J Sleep Res.* 1995; 4:71–77. [PubMed: 10607178]
51. Fletcher EC. Physiological consequences of intermittent hypoxia: Systemic blood pressure. *J Appl Physiol.* 2001; 90:1600–1605. [PubMed: 11247966]
52. Fletcher EC, Lesske J, Behm R, Miller CC III, Stauss H, Unger T. Carotid chemoreceptors, systemic blood pressure, and chronic episodic hypoxia mimicking sleep apnea. *J Appl Physiol.* 1992; 72:1978–1984. [PubMed: 1601808]

53. Fletcher EC, Lesske J, Culman J, Miller CC, Unger T. Sympathetic denervation blocks blood pressure elevation in episodic hypoxia. *Hypertension*. 1992; 20:612–619. [PubMed: 1428112]
54. Fletcher EC, Miller J, Schaaf JW, Fletcher JG. Urinary catecholamines before and after tracheostomy in patients with obstructive sleep apnea and hypertension. *Sleep*. 1987; 10:35–44. [PubMed: 3563246]
55. Garcia-Rio F, Racionero MA, Pino JM, Martinez I, Ortuno F, Villasante C, Villamor J. Sleep apnea and hypertension. *Chest*. 2000; 117:1417–1425. [PubMed: 10807831]
56. Gardner PR. Aconitase: Sensitive target and measure of superoxide. *Methods Enzymol*. 2002; 349:9–23. [PubMed: 11912933]
57. Gauda EB, Carroll JL, Donnelly DF. Developmental maturation of chemosensitivity to hypoxia of peripheral arterial chemoreceptors—Invited article. *Adv Exp Med Biol*. 2009; 648:243–255. [PubMed: 19536487]
58. Gluckman PD, Cutfield W, Hofman P, Hanson MA. The fetal, neonatal, and infant environments—the long-term consequences for disease risk. *Early Hum Dev*. 2005; 81:51–59. [PubMed: 15707715]
59. Gozal E, Shah ZA, Pequignot JM, Pequignot J, Sachleben LR, Czyzyk-Krzeska MF, Li RC, Guo SZ, Gozal D. Tyrosine hydroxylase expression and activity in the rat brain: Differential regulation after long-term intermittent or sustained hypoxia. *J Appl Physiol*. 2005; 99:642–649. [PubMed: 15817718]
60. Greenberg HE, Sica A, Batson D, Scharf SM. Chronic intermittent hypoxia increases sympathetic responsiveness to hypoxia and hypercapnia. *J Appl Physiol*. 1999; 86:298–305. [PubMed: 9887143]
61. Greenberg HE, Sica AL, Scharf SM, Ruggiero DA. Expression of c-fos in the rat brainstem after chronic intermittent hypoxia. *Brain Res*. 1999; 816:638–645. [PubMed: 9878890]
62. Guzy RD, Schumacker PT. Oxygen sensing by mitochondria at complex III: The paradox of increased reactive oxygen species during hypoxia. *Exp Physiol*. 2006; 91:807–819. [PubMed: 16857720]
63. Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: An overview. *Methods Enzymol*. 1990; 186:1–85. [PubMed: 2172697]
64. Hansen J, Sander M. Sympathetic neural overactivity in healthy humans after prolonged exposure to hypobaric hypoxia. *J Physiol*. 2003; 546:921–929. [PubMed: 12563015]
65. Hanson MA. Role of chemoreceptors in effects of chronic hypoxia. *Comp Biochem Physiol A Mol Integr Physiol*. 1998; 119:695–703. [PubMed: 9683408]
66. Heath D, Smith P, Fitch R, Harris P. Comparative pathology of the enlarged carotid body. *J Comp Pathol*. 1985; 95:259–271. [PubMed: 3837795]
67. Hedner J, Ejnell H, Sellgren J, Hedner T, Wallin G. Is high and fluctuating muscle nerve sympathetic activity in the sleep apnoea syndrome of pathogenetic importance for the development of hypertension? *J Hypertens Suppl*. 1988; 6:S529–S531. [PubMed: 3241251]
68. Hedner JA, Wilcox I, Laks L, Grunstein RR, Sullivan CE. A specific and potent pressor effect of hypoxia in patients with sleep apnea. *Am Rev Respir Dis*. 1992; 146:1240–1245. [PubMed: 1443878]
69. Hendricks JC, Kline LR, Kovalski RJ, O'Brien JA, Morrison AR, Pack AI. The English bulldog: A natural model of sleep-disordered breathing. *J Appl Physiol*. 1987; 63:1344–1350. [PubMed: 3693167]
70. Hendricks T, Francis N, Fyodorov D, Deneris ES. The ETS domain factor Pet-1 is an early and precise marker of central serotonin neurons and interacts with a conserved element in serotonergic genes. *J Neurosci*. 1999; 19:10348–10356. [PubMed: 10575032]
71. Hendricks TJ, Fyodorov DV, Wegman LJ, Lelutiu NB, Pehek EA, Yamamoto B, Silver J, Weeber EJ, Sweatt JD, Deneris ES. Pet-1 ETS gene plays a critical role in 5-HT neuron development and is required for normal anxiety-like and aggressive behavior. *Neuron*. 2003; 37:233–247. [PubMed: 12546819]
72. Herman JP, Cullinan WE. Neurocircuitry of stress: Central control of the hypothalamo-pituitary-adrenocortical axis. *Trends Neurosci*. 1997; 20:78–84. [PubMed: 9023876]

73. Heymans C, Bouckaert JJ. Sinus caroticus and respiratory reflexes. *J Physiol.* 1930; 69:254–266. [PubMed: 16994101]
74. Heymans C, Bouckaert JJ, Dautrebande L. Sinus carotidien et reflexes respiratoires: Sensibilite des sinus carotidiens aux substances chimiques. Action stimulante respiratoire reflexe du sulfure de sodium, du cyanure de potassium, de la nicotine et de la lobeline. *Arch Int Pharmacodyn Ther.* 1931; 40:54–91.
75. Hibbs AM, Johnson NL, Rosen CL, Kirchner HL, Martin R, Storfer-Isser A, Redline S. Prenatal and neonatal risk factors for sleep disordered breathing in school-aged children born preterm. *J Pediatr.* 2008; 153:176–182. [PubMed: 18534222]
76. Ho SM, Tang WY, Belmonte de Frausto J, Prins GS. Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. *Cancer Res.* 2006; 66:5624–5632. [PubMed: 16740699]
77. Holmquist-Mengelbier L, Fredlund E, Lofstedt T, Noguera R, Navarro S, Nilsson H, Pietras A, Vallon-Christersson J, Borg A, Gradin K, Poellinger L, Pahlman S. Recruitment of HIF-1alpha and HIF-2alpha to common target genes is differentially regulated in neuroblastoma: HIF-2alpha promotes an aggressive phenotype. *Cancer Cell.* 2006; 10:413–423. [PubMed: 17097563]
78. Hornyak M, Cejnar M, Elam M, Matousek M, Wallin BG. Sympathetic muscle nerve activity during sleep in man. *Brain.* 1991; 114(Pt 3):1281–1295. [PubMed: 2065250]
79. Huang J, Lusina S, Xie T, Ji E, Xiang S, Liu Y, Weiss JW. Sympathetic response to chemostimulation in conscious rats exposed to chronic intermittent hypoxia. *Respir Physiol Neurobiol.* 2009; 166:102–106. [PubMed: 19429526]
80. Hui AS, Striet JB, Gudelsky G, Soukhova GK, Gozal E, Beitner-Johnson D, Guo SZ, Sachleben LR, Haycock JW, Gozal D, Czyzyk-Krzeska MF. Regulation of catecholamines by sustained and intermittent hypoxia in neuroendocrine cells and sympathetic neurons. *Hypertension.* 2003; 42:1130–1136. [PubMed: 14597643]
81. Imadojemu VA, Gleeson K, Gray KS, Sinoway LI, Leuenberger UA. Obstructive apnea during sleep is associated with peripheral vasoconstriction. *Am J Respir Crit Care Med.* 2002; 165:61–66. [PubMed: 11779731]
82. Imadojemu VA, Mawji Z, Kunselman A, Gray KS, Hogeman CS, Leuenberger UA. Sympathetic chemoreflex responses in obstructive sleep apnea and effects of continuous positive airway pressure therapy. *Chest.* 2007; 131:1406–1413. [PubMed: 17494791]
83. Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, Gassmann M, Gearhart JD, Lawler AM, Yu AY, Semenza GL. Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. *Genes Dev.* 1998; 12:149–162. [PubMed: 9436976]
84. Jacono F, Peng Y, Kumar G, Prabhakar N. Modulation of the hypoxic sensory response of the carotid body by 5-hydroxytryptamine: Role of the 5-HT2 receptor. *Respir Physiol Neurobiol.* 2005; 145:135–142. [PubMed: 15705529]
85. Jones PA, Baylin SB. The epigenomics of cancer. *Cell.* 2007; 128:683–692. [PubMed: 17320506]
86. Joyeux-Faure M, Stanke-Labesque F, Lefebvre B, Béguin P, Godin-Ribuot D, Ribaut C, Launois SH, Bessard G, Lévy P. Chronic intermittent hypoxia increases infarction in the isolated rat heart. *J Appl Physiol (1985).* 2005; 98:1691–1696. [PubMed: 15618317]
87. Julien C, Bairam A, Joseph V. Chronic intermittent hypoxia reduces ventilatory long-term facilitation and enhances apnea frequency in new-born rats. *Am J Physiol Regul Integr Comp Physiol.* 2008; 294:R1356–R1366. [PubMed: 18287216]
88. Jyung RW, LeClair EE, Bernat RA, Kang TS, Ung F, McKenna MJ, Tuan RS. Expression of angiogenic growth factors in paragangliomas. *Laryngoscope.* 2000; 110:161–167. [PubMed: 10646734]
89. Kaczmarek E, Bakker JP, Clarke DN, Csizmadia E, Kocher O, Veves A, Tecilazich F, O'Donnell CP, Ferran C, Malhotra A. Molecular biomarkers of vascular dysfunction in obstructive sleep apnea. *PLoS One.* 2013; 8:e70559. [PubMed: 23923005]
90. Kanagy NL, Walker BR, Nelin LD. Role of endothelin in intermittent hypoxia-induced hypertension. *Hypertension.* 2001; 37:511–515. [PubMed: 11230327]
91. Kara T, Narkiewicz K, Somers VK. Chemoreflexes—Physiology and clinical implications. *Acta Physiol Scand.* 2003; 177:377–384. [PubMed: 12609009]

92. Katragadda S, Xie A, Puleo D, Skatrud JB, Morgan BJ. Neural mechanism of the pressor response to obstructive and nonobstructive apnea. *J Appl Physiol* (1985). 1997; 83:2048–2054. [PubMed: 9390980]
93. Khan SA, Nanduri J, Yuan G, Kinsman B, Kumar GK, Joseph J, Kalyanaraman B, Prabhakar NR. NADPH oxidase 2 mediates intermittent hypoxia-induced mitochondrial complex I inhibition: Relevance to blood pressure changes in rats. *Antioxid Redox Signal*. 2011; 14:533–542. [PubMed: 20618070]
94. Kholwadwala D, Donnelly DF. Maturation of carotid chemoreceptor sensitivity to hypoxia: In vitro studies in the newborn rat. *J Physiol*. 1992; 453:461–473. [PubMed: 1464840]
95. Kline DD, Ramirez-Navarro A, Kunze DL. Adaptive depression in synaptic transmission in the nucleus of the solitary tract after in vivo chronic intermittent hypoxia: Evidence for homeostatic plasticity. *J Neurosci*. 2007; 27:4663–4673. [PubMed: 17460079]
96. Kline DD, Takacs KN, Ficker E, Kunze DL. Dopamine modulates synaptic transmission in the nucleus of the solitary tract. *J Neurophysiol*. 2002; 88:2736–2744. [PubMed: 12424308]
97. Knight WD, Little JT, Carreno FR, Toney GM, Mifflin SW, Cunningham JT. Chronic intermittent hypoxia increases blood pressure and expression of FosB/DeltaFosB in central autonomic regions. *Am J Physiol Regul Integr Comp Physiol*. 2011; 301:R131–R139. [PubMed: 21543638]
98. Kumar GK, Rai V, Sharma SD, Ramakrishnan DP, Peng YJ, Souvannakitti D, Prabhakar NR. Chronic intermittent hypoxia induces hypoxia-evoked catecholamine efflux in adult rat adrenal medulla via oxidative stress. *J Physiol*. 2006; 575:229–239. [PubMed: 16777938]
99. Kumar P, Prabhakar NR. Peripheral chemoreceptors: Function and plasticity of the carotid body. *Comp Physiol*. 2012; 2:141–219.
100. Lagercrantz H, Edwards D, Henderson-Smart D, Hertzberg T, Jeffery H. Autonomic reflexes in preterm infants. *Acta Paediatr Scand*. 1990; 79:721–728. [PubMed: 2239264]
101. Lagercrantz H, Sjöquist B. Deficient sympatho-adrenal activity—a cause of apnoea? Urinary excretion of catecholamines and their metabolites in preterm infants. *Early Hum Dev*. 1980; 4:405–409. [PubMed: 7460804]
102. Laidler P, Kay JM. Ultrastructure of carotid body in rats living at a simulated altitude of 4300 metres. *J Pathol*. 1978; 124:27–33. [PubMed: 722370]
103. Lam SY, Leung PS. A locally generated angiotensin system in rat carotid body. *Regul Pept*. 2002; 107:97–103. [PubMed: 12137971]
104. Lam SY, Leung PS. Chronic hypoxia activates a local angiotensin-generating system in rat carotid body. *Mol Cell Endocrinol*. 2003; 203:147–153. [PubMed: 12782411]
105. Lam SY, Liu Y, Ng KM, Lau CF, Liong EC, Tipoe GL, Fung ML. Chronic intermittent hypoxia induces local inflammation of the rat carotid body via functional upregulation of proinflammatory cytokine pathways. *Histochem Cell Biol*. 2012; 137:303–317. [PubMed: 22187044]
106. Lam SY, Liu Y, Ng KM, Liong EC, Tipoe GL, Leung PS, Fung ML. Upregulation of a local renin-angiotensin system in the rat carotid body during chronic intermittent hypoxia. *Exp Physiol*. 2014; 99:220–231. [PubMed: 24036592]
107. Lam SY, Tipoe GL, Liong EC, Fung ML. Hypoxia-inducible factor (HIF)-1 α and endothelin-1 expression in the rat carotid body during intermittent hypoxia. *Adv Exp Med Biol*. 2006; 580:21–27. discussion 351–359. [PubMed: 16683693]
108. Lam SY, Tipoe GL, Liong EC, Fung ML. Differential expressions and roles of hypoxia-inducible factor-1 α , -2 α and -3 α in the rat carotid body during chronic and intermittent hypoxia. *Histol Histopathol*. 2008; 23:271–280. [PubMed: 18072084]
109. Lesske J, Fletcher EC, Bao G, Unger T. Hypertension caused by chronic intermittent hypoxia—Influence of chemoreceptors and sympathetic nervous system. *J Hypertens*. 1997; 15:1593–1603. [PubMed: 9488210]
110. Leuenberger U, Jacob E, Sweer L, Waravdekar N, Zwillich C, Sinoway L. Surges of muscle sympathetic nerve activity during obstructive apnea are linked to hypoxemia. *J Appl Physiol*. 1995; 79:581–588. [PubMed: 7592221]

111. Leuenberger UA, Brubaker D, Quraishi S, Hogeman CS, Imadojemu VA, Gray KS. Effects of intermittent hypoxia on sympathetic activity and blood pressure in humans. *Auton Neurosci*. 2005; 121:87–93. [PubMed: 15996901]
112. Leung PS, Lam SY, Fung ML. Chronic hypoxia upregulates the expression and function of AT(1) receptor in rat carotid body. *J Endocrinol*. 2000; 167:517–524. [PubMed: 11115779]
113. Li YL, Gao L, Zucker IH, Schultz HD. NADPH oxidase-derived super-oxide anion mediates angiotensin II-enhanced carotid body chemoreceptor sensitivity in heart failure rabbits. *Cardiovasc Res*. 2007; 75:546–554. [PubMed: 17499230]
114. Lonergan RP III, Ware JC, Atkinson RL, Winter WC, Suratt PM. Sleep apnea in obese miniature pigs. *J Appl Physiol*. 1998; 84:531–536. [PubMed: 9475862]
115. Lusina SJ, Kennedy PM, Inglis JT, McKenzie DC, Ayas NT, Sheel AW. Long-term intermittent hypoxia increases sympathetic activity and chemosensitivity during acute hypoxia in humans. *J Physiol*. 2006; 575:961–970. [PubMed: 16809359]
116. Machacek DW, Garraway SM, Shay BL, Hochman S. Serotonin 5-HT(2) receptor activation induces a long-lasting amplification of spinal reflex actions in the rat. *J Physiol*. 2001; 537:201–207. [PubMed: 11711573]
117. Makarenko VV, Nanduri J, Raghuraman G, Fox AP, Gadalla MM, Kumar GK, Snyder SH, Prabhakar NR. Endogenous H₂S is required for hypoxic sensing by carotid body glomus cells. *Am J Physiol Cell Physiol*. 2012; 303:C916–C923. [PubMed: 22744006]
118. Manalo DJ, Rowan A, Lavoie T, Natarajan L, Kelly BD, Ye SQ, Garcia JG, Semenza GL. Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood*. 2005; 105:659–669. [PubMed: 15374877]
119. Marcus NJ, Li YL, Bird CE, Schultz HD, Morgan BJ. Chronic intermittent hypoxia augments chemoreflex control of sympathetic activity: Role of the angiotensin II type 1 receptor. *Respir Physiol Neurobiol*. 2010; 171:36–45. [PubMed: 20153844]
120. Marrone O, Riccobono L, Salvaggio A, Mirabella A, Bonanno A, Bonsignore MR. Catecholamines and blood pressure in obstructive sleep apnea syndrome. *Chest*. 1993; 103:722–727. [PubMed: 8449058]
121. Maelshagen J, Sherff CM, Carew TJ. Differential induction of long-term synaptic facilitation by spaced and massed applications of serotonin at sensory neuron synapses of *Aplysia californica*. *Learn Mem*. 1998; 5:246–256. [PubMed: 10454368]
122. McCord JM, Roy RS, Schaffer SW. Free radicals and myocardial ischemia. The role of xanthine oxidase. *Adv Myocardiol*. 1985; 5:183–189. [PubMed: 2982206]
123. Meneshian A, Bulkley GB. The physiology of endothelial xanthine oxidase: From urate catabolism to reperfusion injury to inflammatory signal transduction. *Microcirculation*. 2002; 9:161–175. [PubMed: 12080414]
124. Mitchell GS, Johnson SM. Neuroplasticity in respiratory motor control. *J Appl Physiol*. 2003; 94:358–374. [PubMed: 12486024]
125. Moraes DJ, da Silva MP, Bonagamba LG, Mecawi AS, Zoccal DB, Antunes-Rodrigues J, Varanda WA, Machado BH. Electrophysiological properties of rostral ventrolateral medulla presympathetic neurons modulated by the respiratory network in rats. *J Neurosci*. 2013; 33:19223–19237. [PubMed: 24305818]
126. Morgan BJ, Crabtree DC, Palta M, Skatrud JB. Combined hypoxia and hypercapnia evokes long-lasting sympathetic activation in humans. *J Appl Physiol*. 1995; 79:205–213. [PubMed: 7559221]
127. Nanduri J, Makarenko V, Reddy VD, Yuan G, Pawar A, Wang N, Khan SA, Zhang X, Kinsman B, Peng Y-J, Kumar GK, Fox AP, Godley LA, Semenza GL, Prabhakar NR. Epigenetic regulation of hypoxic sensing disrupts cardiorespiratory homeostasis. *Proc Natl Acad Sci U S A*. 2012; 109:2515–2520. [PubMed: 22232674]
128. Nanduri J, Vaddi DR, Khan SA, Wang N, Makarenko V, Prabhakar NR. Xanthine oxidase mediates hypoxia-inducible factor-2 α degradation by intermittent hypoxia. *PLoS One*. 2013; 8:e75838. [PubMed: 24124516]
129. Nanduri J, Wang N, Yuan G, Khan SA, Souvannakitti D, Peng YJ, Kumar GK, Garcia JA, Prabhakar NR. Intermittent hypoxia degrades HIF-2 α via calpains resulting in oxidative

- stress: Implications for recurrent apnea-induced morbidities. *Proc Natl Acad Sci U S A*. 2009; 106:1199–1204. [PubMed: 19147445]
130. Nanduri J, Yuan G, Kumar GK, Semenza GL, Prabhakar NR. Transcriptional responses to intermittent hypoxia. *Respir Physiol Neurobiol*. 2008; 164:277–281. [PubMed: 18692603]
 131. Narkiewicz K, Somers VK. The sympathetic nervous system and obstructive sleep apnea: Implications for hypertension. *J Hypertens*. 1997; 15:1613–1619. [PubMed: 9488212]
 132. Narkiewicz K, van de Borne PJ, Pesek CA, Dyken ME, Montano N, Somers VK. Selective potentiation of peripheral chemoreflex sensitivity in obstructive sleep apnea. *Circulation*. 1999; 99:1183–1189. [PubMed: 10069786]
 133. Nieto FJ, Young TB, Lind BK, Shahar E, Samet JM, Redline S, D'Agostino RB, Newman AB, Lebowitz MD, Pickering TG. Association of sleep-disordered breathing, sleep apnea, and hypertension in a large community-based study. *Sleep Heart Health Study*. *JAMA*. 2000; 283:1829–1836. [PubMed: 10770144]
 134. Nishino T. The conversion of xanthine dehydrogenase to xanthine oxidase and the role of the enzyme in reperfusion injury. *J Biochem*. 1994; 116:1–6. [PubMed: 7798166]
 135. Nock ML, Difiore JM, Arko MK, Martin RJ. Relationship of the ventilatory response to hypoxia with neonatal apnea in preterm infants. *J Pediatr*. 2004; 144:291–295. [PubMed: 15001929]
 136. Okada H, Iwase S, Mano T, Sugiyama Y, Watanabe T. Changes in muscle sympathetic nerve activity during sleep in humans. *Neurology*. 1991; 41:1961–1966. [PubMed: 1745356]
 137. Ortiz FC, Del Rio R, Varas R, Iturriaga R. Contribution of TASK-like potassium channels to the enhanced rat carotid body responsiveness to hypoxia. *Adv Exp Med Biol*. 2012; 758:365–371. [PubMed: 23080184]
 138. Paavonen EJ, Strang-Karlsson S, Räikkönen K, Heinonen K, Pesonen AK, Hovi P, Andersson S, Järvenpää AL, Eriksson JG, Kajantie E. Very low birth weight increases risk for sleep-disordered breathing in young adulthood: The Helsinki Study of Very Low Birth Weight Adults. *Pediatrics*. 2007; 120:778–784. [PubMed: 17908765]
 139. Pawar A, Nanduri J, Yuan G, Khan SA, Wang N, Kumar GK, Prabhakar NR. Reactive oxygen species-dependent endothelin signaling is required for augmented hypoxic sensory response of the neonatal carotid body by intermittent hypoxia. *Am J Physiol Regul Integr Comp Physiol*. 2009; 296:R735–R742. [PubMed: 19109370]
 140. Pawar A, Peng Y-J, Jacono FJ, Prabhakar NR. Comparative analysis of neonatal and adult rat carotid body responses to chronic intermittent hypoxia. *J Appl Physiol*. 2008; 104:1287–1294. [PubMed: 18187605]
 141. Peng Y-J, Nanduri J, Raghuraman G, Souvannakitti D, Gadalla MM, Kumar GK, Snyder SH, Prabhakar NR. H₂S mediates O₂ sensing in the carotid body. *Proc Natl Acad Sci U S A*. 2010; 107:10719–10724. [PubMed: 20556885]
 142. Peng Y-J, Nanduri J, Zhang X, Wang N, Raghuraman G, Seagard J, Kumar GK, Prabhakar NR. Endothelin-1 mediates attenuated carotid baroreceptor activity by intermittent hypoxia. *J Appl Physiol*. 2012; 112:187–196. [PubMed: 22016368]
 143. Peng YJ, Makarenko VV, Nanduri J, Vasavda C, Raghuraman G, Yuan G, Gadalla MM, Kumar GK, Snyder SH, Prabhakar NR. Inherent variations in CO-H₂S-mediated carotid body O₂ sensing mediate hypertension and pulmonary edema. *Proc Natl Acad Sci U S A*. 2014; 111:1174–1179. [PubMed: 24395806]
 144. Peng YJ, Nanduri J, Khan SA, Yuan G, Wang N, Kinsman B, Vaddi DR, Kumar GK, Garcia JA, Semenza GL, Prabhakar NR. Hypoxia-inducible factor 2alpha (HIF-2alpha) heterozygous-null mice exhibit exaggerated carotid body sensitivity to hypoxia, breathing instability, and hypertension. *Proc Natl Acad Sci U S A*. 2011; 108:3065–3070. [PubMed: 21288809]
 145. Peng YJ, Nanduri J, Raghuraman G, Wang N, Kumar GK, Prabhakar NR. Role of oxidative stress-induced endothelin-converting enzyme activity in the alteration of carotid body function by chronic intermittent hypoxia. *Exp Physiol*. 2013; 98:1620–1630. [PubMed: 23913764]
 146. Peng YJ, Nanduri J, Yuan G, Wang N, Deneris E, Pendyala S, Natarajan V, Kumar GK, Prabhakar NR. NADPH oxidase is required for the sensory plasticity of the carotid body by chronic intermittent hypoxia. *J Neurosci*. 2009; 29:4903–4910. [PubMed: 19369559]

147. Peng YJ, Overholt JL, Kline D, Kumar GK, Prabhakar NR. Induction of sensory long-term facilitation in the carotid body by intermittent hypoxia: Implications for recurrent apneas. *Proc Natl Acad Sci U S A*. 2003; 100:10073–10078. [PubMed: 12907705]
148. Peng YJ, Prabhakar NR. Effect of two paradigms of chronic intermittent hypoxia on carotid body sensory activity. *J Appl Physiol*. 2004; 96:1236–1242. discussion 1196. [PubMed: 14660510]
149. Peng YJ, Raghuraman G, Khan SA, Kumar GK, Prabhakar NR. Angiotensin II evokes sensory long-term facilitation of the carotid body via NADPH oxidase. *J Appl Physiol*. 2011; 111:964–970. [PubMed: 21636565]
150. Peng YJ, Rennison J, Prabhakar NR. Intermittent hypoxia augments carotid body and ventilatory response to hypoxia in neonatal rat pups. *J Appl Physiol*. 2004; 97:2020–2025. [PubMed: 15258129]
151. Peng YJ, Yuan G, Jacono FJ, Kumar GK, Prabhakar NR. 5-HT evokes sensory long-term facilitation of rodent carotid body via activation of NADPH oxidase. *J Physiol*. 2006; 576:289–295. [PubMed: 16887872]
152. Peng YJ, Yuan G, Khan S, Nanduri J, Makarenko VV, Reddy VD, Vasavda C, Kumar GK, Semenza GL, Prabhakar NR. Regulation of hypoxia inducible factor- α isoforms and redox state by carotid body neural activity in rats. *J Physiol*. 2014; 592:3841–3858. [PubMed: 24973414]
153. Peng YJ, Yuan G, Ramakrishnan D, Sharma SD, Bosch-Marce M, Kumar GK, Semenza GL, Prabhakar NR. Heterozygous HIF-1 α deficiency impairs carotid body-mediated systemic responses and reactive oxygen species generation in mice exposed to intermittent hypoxia. *J Physiol*. 2006; 577:705–716. [PubMed: 16973705]
154. Pépin JL, Tamié R, Barone-Rochette G, Launois SH, Lévy P, Baguet JP. Comparison of continuous positive airway pressure and valsartan in hypertensive patients with sleep apnea. *Am J Respir Crit Care Med*. 2010; 182:954–960. [PubMed: 20522795]
155. Peppard PE, Young T, Palta M, Skatrud J. Prospective study of the association between sleep-disordered breathing and hypertension. *N Engl J Med*. 2000; 342:1378–1384. [PubMed: 10805822]
156. Pequignot JM, Hellstrom S, Johansson C. Intact and sympathectomized carotid bodies of long-term hypoxic rats: A morphometric ultrastructural study. *J Neurocytol*. 1984; 13:481–493. [PubMed: 6481409]
157. Pinilla PJ, Hernandez AT, Camello MC, Pozo MJ, Toescu EC, Camello PJ. Non-stimulated Ca^{2+} leak pathway in cerebellar granule neurones. *Biochem Pharmacol*. 2005; 70:786–793. [PubMed: 16018974]
158. Poets CF, Samuels MP, Southall DP. Epidemiology and pathophysiology of apnoea of prematurity. *Biol Neonate*. 1994; 65:211–219. [PubMed: 8038285]
159. Prabhakar NR. Oxygen sensing by the carotid body chemoreceptors. *J Appl Physiol*. 2000; 88:2287–2295. [PubMed: 10846047]
160. Prabhakar NR. Oxygen sensing during intermittent hypoxia: Cellular and molecular mechanisms. *J Appl Physiol*. 2001; 90:1986–1994. [PubMed: 11299293]
161. Prabhakar NR. Sensory plasticity of the carotid body: Role of reactive oxygen species and physiological significance. *Respir Physiol Neurobiol*. 2011; 178:375–380. [PubMed: 21621009]
162. Prabhakar NR, Peng Y-H, Kumar GK, Pawar A. Altered carotid body function by intermittent hypoxia in neonates and adults: Relevance to recurrent apneas. *Respir Physiol Neurobiol*. 2007; 157:148–153. [PubMed: 17317339]
163. Prabhakar NR, Semenza GL. Adaptive and maladaptive cardiorespiratory responses to continuous and intermittent hypoxia mediated by hypoxia-inducible factors 1 and 2. *Physiol Rev*. 2012; 92:967–1003. [PubMed: 22811423]
164. Reeves SR, Gozal E, Guo SZ, Sachleben LR Jr, Brittan KR, Lipton AJ, Gozal D. Effect of long-term intermittent and sustained hypoxia on hypoxic ventilatory and metabolic responses in the adult rat. *J Appl Physiol*. 2003; 95:1767–1774. [PubMed: 14555663]
165. Rey S, Corthorn J, Chacon C, Iturriaga R. Expression and immunolocalization of endothelin peptides and its receptors, ETA and ETB, in the carotid body exposed to chronic intermittent hypoxia. *J Histochem Cytochem*. 2007; 55:167–174. [PubMed: 17046837]

166. Rey S, Del Rio R, Alcaayaga J, Iturriaga R. Chronic intermittent hypoxia enhances cat chemosensory and ventilatory responses to hypoxia. *J Physiol.* 2004; 560:577–586. [PubMed: 15319419]
167. Rey S, Del Rio R, Iturriaga R. Contribution of endothelin-1 to the enhanced carotid body chemosensory responses induced by chronic intermittent hypoxia. *Brain Res.* 2006; 1086:152–159. [PubMed: 16595126]
168. Rey S, Del Rio R, Iturriaga R. Contribution of endothelin-1 and endothelin A and B receptors to the enhanced carotid body chemosensory responses induced by chronic intermittent hypoxia. *Adv Exp Med Biol.* 2008; 605:228–232. [PubMed: 18085277]
169. Rosen CL, Larkin EK, Kirchner HL, Emancipator JL, Bivins SF, Surovec SA, Martin RJ, Redline S. Prevalence and risk factors for sleep-disordered breathing in 8- to 11-year-old children: Association with race and prematurity. *J Pediatr.* 2003; 142:383–389. [PubMed: 12712055]
170. Saarelainen S, Seppala E, Laasonen K, Hasan J. Circulating endothelin-1 in obstructive sleep apnea. *Endothelium.* 1997; 5:115–118. [PubMed: 9237045]
171. Salman S, Buttigieg J, Nurse CA. Ontogeny of O₂ and CO₂/H⁺ chemosensitivity in adrenal chromaffin cells: Role of innervation. *J Exp Biol.* 2014; 217:673–681. [PubMed: 24574383]
172. Santos F, Dean W. Epigenetic reprogramming during early development in mammals. *Reproduction.* 2004; 127:643–651. [PubMed: 15175501]
173. Sawchenko PE, Brown ER, Chan RK, Ericsson A, Li HY, Roland BL, Kovacs KJ. The paraventricular nucleus of the hypothalamus and the functional neuroanatomy of visceromotor responses to stress. *Prog Brain Res.* 1996; 107:201–222. [PubMed: 8782521]
174. Semenza GL. HIF-1: Mediator of physiological and pathophysiological responses to hypoxia. *J Appl Physiol.* 2000; 88:1474–1480. [PubMed: 10749844]
175. Shahar E, Whitney CW, Redline S, Lee ET, Newman AB, Javier Nieto F, O'Connor GT, Boland LL, Schwartz JE, Samet JM. Sleep-disordered breathing and cardiovascular disease: Cross-sectional results of the Sleep Heart Health Study. *Am J Respir Crit Care Med.* 2001; 163:19–25. [PubMed: 11208620]
176. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. *Carcinogenesis.* 2010; 31:27–36. [PubMed: 19752007]
177. Shoemaker JK, Vovk A, Cunningham DA. Peripheral chemoreceptor contributions to sympathetic and cardiovascular responses during hypercapnia. *Can J Physiol Pharmacol.* 2002; 80:1136–1144. [PubMed: 12564639]
178. Sica AL, Greenberg HE, Scharf SM, Ruggiero DA. Immediate-early gene expression in cerebral cortex following exposure to chronicintermittent hypoxia. *Brain Res.* 2000; 870:204–210. [PubMed: 10869520]
179. Silva AQ, Schreihof AM. Altered sympathetic reflexes and vascular reactivity in rats after exposure to chronic intermittent hypoxia. *J Physiol.* 2011; 589:1463–1476. [PubMed: 21242253]
180. Somers VK, Abboud FM. Chemoreflexes—Responses, interactions and implications for sleep apnea. *Sleep.* 1993; 16:S30–S33. discussion S33–S34. [PubMed: 8178019]
181. Somers VK, Dyken ME, Clary MP, Abboud FM. Sympathetic neural mechanisms in obstructive sleep apnea. *J Clin Invest.* 1995; 96:1897–1904. [PubMed: 7560081]
182. Somers VK, Dyken ME, Mark AL, Abboud FM. Sympathetic-nerve activity during sleep in normal subjects. *N Engl J Med.* 1993; 328:303–307. [PubMed: 8419815]
183. Souvannakitti D, Kumar GK, Fox A, Prabhakar NR. Neonatal intermittent hypoxia leads to long-lasting facilitation of acute hypoxia-evoked catecholamine secretion from rat chromaffin cells. *J Neurophysiol.* 2009; 101:2837–2846. [PubMed: 19339466]
184. Souvannakitti D, Nanduri J, Yuan G, Kumar GK, Fox AP, Prabhakar NR. NADPH oxidase-dependent regulation of T-type Ca²⁺ channels and ryanodine receptors mediate the augmented exocytosis of catecholamines from intermittent hypoxia-treated neonatal rat chromaffin cells. *J Neurosci.* 2010; 30:10763–10772. [PubMed: 20705601]
185. Sterni LM, Bamford OS, Wasicko MJ, Carroll JL. Chronic hypoxia abolished the postnatal increase in carotid body type I cell sensitivity to hypoxia. *Am J Physiol.* 1999; 277:L645–L652. [PubMed: 10484473]

186. Stoohs R, Guilleminault C. Cardiovascular changes associated with obstructive sleep apnea syndrome. *J Appl Physiol.* 1992; 72:583–589. [PubMed: 1559936]
187. Suh YA, Arnold RS, Lassegue B, Shi J, Xu X, Sorescu D, Chung AB, Griendling KK, Lambeth JD. Cell transformation by the superoxide-generating oxidase Mox1. *Nature.* 1999; 401:79–82. [PubMed: 10485709]
188. Suzuki YJ, Jain V, Park AM, Day RM. Oxidative stress and oxidant signaling in obstructive sleep apnea and associated cardiovascular diseases. *Free Radic Biol Med.* 2006; 40:1683–1692. [PubMed: 16678006]
189. Tafil-Klawe M, Thiele AE, Raschke F, Mayer J, Peter JH, von Wichert W. Peripheral chemoreceptor reflex in obstructive sleep apnea patients; a relationship between ventilatory response to hypoxia and nocturnal bradycardia during apnea events. *Pneumologie.* 1991; 45(Suppl 1):309–311. [PubMed: 1866415]
190. Takeuchi Y, Mochizuki-Oda N, Yamada H, Kurokawa K, Watanabe Y. Nonneurogenic hypoxia sensitivity in rat adrenal slices. *Biochem Biophys Res Commun.* 2001; 289:51–56. [PubMed: 11708775]
191. Tamisier R, Pepin JL, Remy J, Baguet JP, Taylor JA, Weiss JW, Levy P. 14 nights of intermittent hypoxia elevate daytime blood pressure and sympathetic activity in healthy humans. *Eur Respir J.* 2011; 37:119–128. [PubMed: 20525723]
192. Thompson RJ, Jackson A, Nurse CA. Developmental loss of hypoxic chemosensitivity in rat adrenomedullary chromaffin cells. *J Physiol.* 1997; 498(Pt 2):503–510. [PubMed: 9032697]
193. Tian H, McKnight SL, Russell DW. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. *Genes Dev.* 1997; 11:72–82. [PubMed: 9000051]
194. Veasey S. Insight from animal models into the cognitive consequences of adult sleep-disordered breathing. *ILAR J.* 2009; 50:307–311. [PubMed: 19506315]
195. Xie A, Skatrud JB, Puleo DS, Morgan BJ. Exposure to hypoxia produces long-lasting sympathetic activation in humans. *J Appl Physiol.* 2001; 91:1555–1562. [PubMed: 11568136]
196. Yu AY, Shimoda LA, Iyer NV, Huso DL, Sun X, McWilliams R, Beaty T, Sham JS, Wiener CM, Sylvester JT, Semenza GL. Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1 α . *J Clin Invest.* 1999; 103:691–696. [PubMed: 10074486]
197. Yuan G, Adhikary G, McCormick AA, Holcroft JJ, Kumar GK, Prabhakar NR. Role of oxidative stress in intermittent hypoxia-induced immediate early gene activation in rat PC12 cells. *J Physiol.* 2004; 557:773–783. [PubMed: 15107478]
198. Yuan G, Khan SA, Luo W, Nanduri J, Semenza GL, Prabhakar NR. Hypoxia-inducible factor 1 mediates increased expression of NADPH oxidase-2 in response to intermittent hypoxia. *J Cell Physiol.* 2011; 226:2925–2933. [PubMed: 21302291]
199. Yuan G, Nanduri J, Bhasker CR, Semenza GL, Prabhakar NR. Ca²⁺/calmodulin kinase-dependent activation of hypoxia inducible factor 1 transcriptional activity in cells subjected to intermittent hypoxia. *J Biol Chem.* 2005; 280:4321–4328. [PubMed: 15569687]
200. Yuan G, Nanduri J, Khan S, Semenza GL, Prabhakar NR. Induction of HIF-1 α expression by intermittent hypoxia: Involvement of NADPH oxidase, Ca²⁺ signaling, prolyl hydroxylases, and mTOR. *J Cell Physiol.* 2008; 217:674–685. [PubMed: 18651560]
201. Zhang W, Mifflin SW. Excitatory amino acid receptors within NTS mediate arterial chemoreceptor reflexes in rats. *Am J Physiol.* 1993; 265:H770–H773. [PubMed: 8368379]
202. Zoccal DB, Bonagamba LG, Oliveira FR, Antunes-Rodrigues J, Machado BH. Increased sympathetic activity in rats submitted to chronic intermittent hypoxia. *Exp Physiol.* 2007; 92:79–85. [PubMed: 17085676]
203. Zoccal DB, Huidobro-Toro JP, Machado BH. Chronic intermittent hypoxia augments sympatho-excitatory response to ATP but not to L-glutamate in the RVLM of rats. *Auton Neurosci.* 2011; 165:156–162. [PubMed: 21684220]
204. Zoccal DB, Simms AE, Bonagamba LG, Braga VA, Pickering AE, Paton JF, Machado BH. Increased sympathetic outflow in juvenile rats submitted to chronic intermittent hypoxia

correlates with enhanced expiratory activity. J Physiol. 2008; 586:3253–3265. [PubMed: 18450774]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1.

The *ex vivo* carotid body responses to acute hypoxia (at black bar in *left panels*) and to acute intermittent hypoxia (AIH; at arrows in *right panels*) in control and rats exposed to 10 days of intermittent hypoxia (IH). pO_2 = partial pressure of O_2 in the medium irrigating the carotid body. *Insets*: represent superimposed action potentials of “single” fiber from which the data were derived. Note the augmented hypoxic sensory response and long-lasting increase in baseline activity (sensory LTF) following AIH in IH-exposed carotid bodies. [From Ref. (162) with permission.]

Figure 2.

Effect of bosentan (50 $\mu\text{mol/L}$) on mild hypoxia-evoked chemosensory discharges from a single control (A) and IH-treated carotid body (B). Summary of the effect of bosentan on chemosensory responses elicited by mild and severe hypoxia in control (C) and IH-treated carotid bodies (D) ($n = 5$ in each group). f_x , frequency of chemosensory discharges expressed in Hz (A and B) or as percent of hypoxic responses in the absence of bosentan. Open bars represent the effects of hypoxia in the absence of bosentan; hashed and closed bars represent the effects of mild and severe hypoxia in the presence of bosentan,

respectively. $*P < 0.05$; $\ddagger P < 0.001$, hypoxic responses following bosentan *versus* hypoxic responses without bosentan. [From Ref. (167) with permission.]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 3.

Schematic illustration of molecular and cellular mechanisms underlying the effects of intermittent hypoxia (IH) on the carotid body and chemoreflex-dependent blood pressure changes. *Keys:* HIF-1 α and HIF-2 α , Hypoxia-inducible factor 1 and 2 α , respectively; Nox2, NADPH oxidase 2; Sod2, Superoxide dismutase 2; ROS, reactive oxygen species; LTF, long-term facilitation.

Figure 4.

Intermittent hypoxia (IH) augments carotid body response to hypoxia in neonatal rats. Examples of carotid body responses to hypoxia in rat pups exposed to 10 days of IH or to normoxia (Control) are shown. Black bars denote the duration of the hypoxic stimulus. Hypoxia: medium $pO_2 = 33$ mmHg. *Insets*: superimposed action potential from a single unit. imp/s, impulses per second. [Modified from Ref. (140) with permission].

Figure 5.

Schematic illustration of epigenetic mechanisms involving DNA hypermethylation of antioxidant enzymes on neonatal intermittent hypoxia-induced hypertension in adults.

Table 1**Intermittent Hypoxia Paradigm-Dependent Changes in Blood Pressure (BP) in Rats**

IH paradigm	Method of BP measurement	Dynamics of BP changes	Reference(s)
3%-5% nadir ambient O ₂ every 30 s, 7 h per day for 35 days	Both tail cuff and femoral arterial catheters	Increase in BP after 35 days	(52)
N ₂ -CO ₂ mixture for 90 s (to generate 5% O ₂ -5% CO ₂) followed by 90 s of compressed air (to achieve 21% O ₂ -0% CO ₂)	Arterial catheters	Increase in BP after 7 days	(90)
10% and 21% O ₂ , for 90 s each, during daylight hours	Telemetry	Increase in BP after 30 days	(80)
40 s with 5% O ₂ and 20 s with 21% O ₂ ; 8 h during daytime for 35 days	Femoral artery	Increase in BP after 5 weeks	(86)
15 s of 5% O ₂ and 5 min of room air, 9 episodes per h and 8 h per day for 10 days	Tail-cuff	Increase in BP after 7 days	(98)
5 min of 21% O ₂ and 4 min of N ₂ ; 8 h per day for 35 days	Catheters placed into the abdominal aorta	Increase in BP after 35 days	(202)
10% and 21% O ₂ every 75 s, 80 cycles per day	Telemetry	Increase in BP after 7 days	(97)
40 s at 6% O ₂ every 9 min, 8 h per day	Catheters placed into the femoral artery and vein	Increase in BP after 2 weeks	(179)