Mechanisms of Sympathetic Regulation in Cardiovascular Disease

Sympatho-adrenal activation by chronic intermittent hypoxia

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Recurrence of apnea with chronic intermittent hypoxia (CIH) is a major clinical problem in adult humans and infants born preterm. Patients with recurrent apnea exhibit heightened sympathetic activity as well as elevated plasma catecholamine levels, and these phenotypes are effectively recapitulated in rodent models of CIH. This article summarizes findings from studies addressing sympathetic activation in recurrent apnea patients and rodent models of CIH and the underlying cellular and molecular mechanisms. Available evidence suggests that augmented chemoreflex and attenuated baroreflex contribute to sympathetic activation by CIH. Studies on rodents showed that CIH augments the carotid body response to hypoxia and attenuates the carotid baroreceptor response to increased sinus pressures. Processing of afferent information from chemoreceptors at the central nervous system is also facilitated by CIH. Adult and neonatal rats exposed to CIH exhibit augmented catecholamine secretion from the adrenal medulla. Adrenal demedullation prevents the elevation of circulating catecholamines in CIH-exposed rodents. Reactive oxygen species (ROS)-mediated signaling is emerging as the major cellular mechanism triggering sympatho-adrenal activation by CIH. Molecular mechanisms underlying increased ROS generation by CIH seem to involve transcriptional dysregulation of genes encoding pro- and antioxidant enzymes by hypoxia-inducible factor-1 and -2, respectively.

carotid chemoreceptor; carotid baroreceptor; sleep apneas; catecholamine secretion; reactive oxygen species; hypoxia-inducible factors

Recurrence of apnea is one of the most commonly occurring breathing disorders in adult humans and infants born preterm (45, 55). Apnea, i.e., cessation of breathing, can occur because of obstruction of the upper airway [obstructive sleep apnea (OSA)] or as a result of defective respiratory rhythm generation by the central nervous system (CNS; central sleep apnea). Human subjects with recurrent apnea exhibit elevated sympathetic nerve activity and increased circulating catecholamine levels (4, 16, 17, 22, 36, 38, 67). Apneas are associated with periodic decreases in arterial blood oxygen (O2) levels, resulting in chronic intermittent hypoxia (CIH). Studies on rodents demonstrated that exposure to CIH increases sympathetic activity similar to that seen in recurrent apnea patients [see Prabhakar et al. (56)], and they further provided important insights into the underlying mechanisms. In this article, we summarize findings from studies addressing the effects of CIH on sympathetic activity and the underlying cellular and molecular mechanisms.

Sympathetic activity in human subjects with recurrent apnea

Normal human subjects, during sleep, have low levels of sympathetic activity and reduced heart rate and blood pressures (26, 46, 68), and these sleep-state-dependent phenotypes are absent in OSA patients (43). Furthermore, adult patients with OSA exhibit elevated sympathetic activity during daytime, wherein apneas are absent (4, 22, 36, 67), and treatment with continuous positive airway pressure (CPAP) lowers sympathetic activity in these subjects (4, 28, 67). Circulating and urinary catecholamines (both norepinephrine and epinephrine) are elevated in OSA subjects (4, 16, 17, 38, 67), and CPAP treatment restores them to normal levels (71). However, whether the elevated plasma catecholamine levels arise from their release, either from the vasculature or from the adrenal medulla in response to increased sympathetic activity, has not been established.

Sympathetic activity in rodent models of CIH

Several factors besides CIH can potentially contribute to sympathetic activation in OSA subjects, including chronic intermittent hypercapnia, sleep fragmentation, changes in tho-
racic pressure, and the ensuing hemodynamic changes during obstruction of the upper airway. However, a major advance in the field of apnea research was the demonstration that exposing rodents to CIH alone is sufficient to elicit sympathetic activation (15). Rats exposed to alternating cycles of hypoxia (30 s of hypoxia and 30 s of normoxia), 8 h/day for 30 days, showed elevated cervical sympathetic nerve activity (20). Other investigators reported similar increases in renal (27), splanchnic (13), thoracic (82), and lumbar (37) sympathetic nerve activities in CIH-exposed rats, albeit using different intermittent hypoxia (IH) paradigms and duration of CIH exposures. Dick et al. (13) reported that the increased sympathetic nerve activity by acute IH is reflected in the late expiratory phase of respiration. This finding was subsequently confirmed in juvenile rats exposed to CIH for 10 days (82), suggesting that CIH alters the coupling of sympathetic-respiratory outputs at the CNS. A recent study by Silva and Schreihofer (65) showed that sympathetic activation evoked by stimulation of the sciatic nerve and the nasal mucosa was more pronounced in rats exposed to 2 wk of CIH than in controls, indicating hypersensitivity of the sympathetic nervous system to a variety of stimuli. Taken together, these studies demonstrate that rats exposed to CIH exhibit sympathetic activation similar to those reported in human subjects with recurrent apnea.

**Effects of CIH on arterial chemoreflex and carotid chemoreceptor activity.** Arterial chemoreflex is a major regulator of the sympathetic activity. The following lines of evidence suggest that the arterial chemoreflex is augmented in OSA subjects: 1) brief hypoxic exposure, which inhibits chemoreceptor activity, reduces blood pressure in OSA patients but not in control subjects (44); 2) hypoxic ventilatory response (HVR), a hallmark response of the chemoreflex, is augmented in OSA subjects compared with controls (23); and 3) activation of muscle sympathetic nerve activity by apneas is more pronounced in OSA subjects compared with controls (66).

Arterial chemoreflex is also augmented in experimental animals exposed to CIH, as evidenced by augmented HVR in cats exposed to 4 days of CIH (61) and in mice exposed to CIH for 10 days (54). Rats exposed to CIH exhibit exaggerated sympathetic nerve responses to hypoxia (3, 27, 37, 82), and this effect was abolished after chronic bilateral sectioning of sinus nerves (15, 56, 57).

The effect of CIH on the carotid body, the primary sensory organ, which initiates the chemoreflex, was examined initially in rats and mice. CIH produces two major effects on the carotid body: 1) enhances the response to acute hypoxia and 2) progressively increases baseline sensory activity following repetitive hypoxia, a phenomenon termed as sensory long-term facilitation (sLTF) (48, 49, 51-53). Subsequent studies in cats exposed to several days of CIH also showed a similar enhanced response of the carotid body to hypoxia (61), suggesting no apparent species differences. Characterization of the carotid body responses further revealed that the effects of CIH develop over time without any morphological changes in the glomus tissue and can be reversed by cessation of CIH (51). The effects of CIH were seen in ex vivo carotid body preparations, suggesting that the effects of CIH are due to a direct action on the chemoreceptor tissue per se and not secondary to cardiovascular changes caused by CIH (51).

Recent studies have shown further that CIH-induced hypersensitivity to hypoxia and sLTF of the carotid body involve distinct neurotransmitters/modulators. For instance, endothelin-1 (ET-1), which is up-regulated by CIH in glomus cells via activation of the ET-1A receptor, mediates the augmented hypoxic sensitivity (47, 61) but not the sLTF induced by CIH (unpublished observations). On the other hand, recent studies suggest that 5-hydroxytryptamine (49) and angiotensin II (53) contribute to CIH-induced sLTF. It has been proposed that an exaggerated carotid body response to hypoxia may account for the pronounced sympathetic activation induced by apnea (66), whereas the sLTF may mediate the daytime increase in sympathetic activity reported in OSA subjects.

**Effects of CIH on arterial baroreflex and carotid baroreceptor activity.** Arterial baroreflex exerts tonic inhibitory influence on sympathetic activity. Lai et al. (35) were the first to report that CIH attenuates baroreflex function in conscious rats, as evidenced by spectral analysis of heart-rate responses. Recently, Peng et al. (50) examined the effects of CIH on arterial baroreflex function and carotid baroreceptor activity in adult rats. They found that CIH decreases baroreflex control of sympathetic nerve activity, as evidenced by reduced inhibition of splanchnic nerve responses to phenylephrine (PE). These investigators reported further that CIH markedly attenuates carotid baroreceptor activity in response to increased carotid sinus pressure. The attenuated carotid baroreceptor activity by CIH was attributed to upregulation of ET-1 in endothelial cells of the carotid sinus region and activation of ET_A receptors (50). However, Gu et al. (21) reported no significant alteration in aortic baroreceptor activity in Fischer 344 rats exposed to CIH.

In contrast to the effects of CIH on baroreflex regulation of splanchnic nerve activity, cervical sympathetic nerve response to PE was unaffected in adult rats exposed to 35 days of CIH (20). Likewise, Machado and his coworkers (80), with the use of a working-heart brain stem preparation, reported an increase in baroreflex function in CIH-exposed juvenile rats. These findings suggest that the effects of CIH on baroreflex regulation of sympathetic activity differ between splanchnic and cervical sympathetic nerves as well as anesthetized vs. decerebrate working-heart brain stem preparations.

Unlike the studies with rodent models of CIH, patients with OSA exhibit downregulation of baroreflex function, as evidenced by attenuated heart rate and vascular responses to baroreceptor activation (2, 10, 39), and CPAP treatment restores baroreflex function (2). Thus it seems reasonable to conclude that CIH attenuates arterial baroreflex function, which is, in part, due to reduced carotid baroreceptor activity. It is interesting to note that upregulation of ET-1 by CIH on one hand depresses baroreceptor responses to increased sinus pressures, whereas on the other hand, it mediates the hypersensitivity of the carotid body to hypoxia. The strikingly opposite effects of CIH on the chemo- and baroreflex functions and their consequences on the sympathetic activity are shown in Fig. 1.

**Effects of CIH on the CNS-regulating sympathetic activity.** Processing of sensory information from the arterial chemo- and baroreceptors at the CNS is critical for the translation of these inputs to appropriate reflex changes in the sympathetic motor output. The dorsal and medial subnuclei of the nucleus tractus solitarius (NTS), including the commissural part of the NTS (cNTS), receive inputs from the carotid sinus nerve (6, 79). The effects of CIH on neuronal activation in the brain stem
regions associated with the sympathetic regulation were assessed by monitoring the protein expressions of c-fos and fosB. Rats exposed to short-term CIH (for 7 days) exhibited increases in FosB/H9004 FosB expression in NTS and rostral ventrolateral medulla (RVLM) (32). On the other hand, c-fos expression was increased in the dorsal and medial subnuclei of the NTS following exposure to 30 days of CIH (64). These findings indicate that both short- and long-term exposures to CIH result in the activation of brain stem neurons associated with the regulation of spinal sympathetic preganglionic neuronal activity.

Neuronal activity in cNTS is regulated by various neurotransmitters, including glutamate (Glu), an excitatory amino acid transmitter, and dopamine (DA), an inhibitory biogenic amine. CIH upregulates N-methyl-D-aspartate receptor 1 (NMDA-R1) expression in the dorsocaudal brain stem (60), Glu receptor types 2/3 subunit expression in cNTS (11), and AMPA- and NMDA-mediated currents in NTS neurons (12). On the other hand, CIH downregulates tyrosine hydroxylase expression in dorsal medulla, the rate-limiting enzyme in DA synthesis (31), it is likely that CIH, by downregulating the synthesis of DA, enhances glutamatergic excitatory transmission in NTS (5). Studies by Kline et al. (30) showed that CIH not only increases postsynaptic cell activity in NTS but also attenuates synaptic transmission between sensory afferents and NTS second-order neurons. This effect seems to occur via reduced transmitter release involving calcium/calmodulin-dependent kinase II activation (30).

Neurons in NTS relay chemoafferent information to the hypothalamic paraventricular nucleus (PVN) and brain stem sympathoexcitatory sites located in the RVLM (24, 62). An earlier study by Greenberg et al. (20) showed that CIH alters neuronal activity of the ventrolateral medulla (A1 noradrenergic cells). Zoccal et al. (81) reported that CIH-induced sympathetic activation is mediated by enhanced purinergic but not glutamatergic transmission in RVLM of juvenile rats. On the other hand, Silva and Schreihofer (65) found that glutamatergic transmission in RVLM is critical for the augmented sympathetic activation by CIH in adult rats. These findings implicate that age is an important variable that determines the type of neurotransmitter(s) mediating the effects of CIH on sympathetic activation by RVLM. A recent study by Coleman et al. (8) reported that PVN neurons in CIH-exposed mice exhibit decreased NMDA-R-mediated currents, reduced NO production by NMDA, and downregulation of NMDA-NR1 receptors in neuronal nitric oxide synthase-positive neurons. Collectively, these studies indicate that the exaggerated chemoreflex-mediated sympathetic activation by CIH involves reconfiguration of neurotransmitter profiles in the CNS.

EFFECTS OF CIH ON THE ADRENAL MEDULLA

The adrenal medulla is one of the major sources of circulating catecholamines. Bao et al. (1) reported that adrenal demedullation prevents a CIH-induced increase in blood pressure and circulating catecholamine levels. Recent studies examined the effects of CIH on catecholamine secretion from the adrenal medulla in adult (33) and neonatal rats (69). Hypoxia-evoked catecholamine secretion from the adrenal medulla was markedly potentiated in adult and neonatal rats by CIH. Studies on mouse adrenal medullary chromaffin cells (AMC) showed that CIH increases the readily releasable pool of secretory vesicles via activa-
tion of PKC (34). Further analysis on neonatal rat AMC showed that the augmented catecholamine secretion by hypoxia by CIH involves activation of calcium ion (Ca\(^{2+}\)) influx via low-threshold T-type Ca\(^{2+}\) channels, as well as mobilization of intracellular Ca\(^{2+}\) stores by ryanodine receptor (RyR) activation (70). These studies suggest that the adrenal medulla contributes to increased plasma catecholamines by CIH.

**REACTIVE OXYGEN SPECIES—A MAJOR CELLULAR MECHANISM MEDIATING THE EFFECTS OF CIH**

Studies on experimental models of CIH. Reactive oxygen species (ROS) signaling is an important cellular mechanism mediating the systemic effects of CIH (55a). CIH increases ROS generation in the carotid body (51), adrenal medulla (33), brain stem (59, 63), and the carotid sinus region (50). Systemic administration of ROS scavengers—e.g., manganese (III) tetraakis (1-methyl-4-pyridyl) porphyrin pentachloride, an O\(_2\) scavenger, and N-acetyl-cysteine, a precursor of glutathione—prevented CIH-induced: 1) hypoxic sensitivity and sLTF of the carotid body, as well as the augmented chemoreflex (51, 52), 2) decreases in carotid baroreceptor activity and baroreflex responses (50), and 3) increases in catecholamine secretion from the adrenal medulla (33, 69), as well as plasma catecholamine levels (33).

Studies on cell cultures and rodent models have identified two major sources of ROS generation by CIH: 1) NADPH oxidases (Nox), especially the Nox2 isoformal (29, 47, 49, 70, 77, 78), and 2) inhibition of the electron transport chain at the mitochondrial complex I (29, 51, 75). A study by Khan et al. (29) showed that ROS generated by Nox2 inhibit the mitochondrial complex I via increased S-glutathionylation of the complex I subunits, resulting in increased mitochondrial ROS generation (i.e., ROS-induced ROS). In addition, CIH downregulated antioxidant enzymes in the carotid body and adrenal medulla (41). These studies demonstrate that both upregulation of pro-oxidant enzymes and downregulation of antioxidant enzymes contribute to increased ROS generation by CIH.

**Cellular targets of CIH-induced ROS.** In the carotid body, ROS mediate the CIH-induced, enhanced hypoxic sensitivity by transcriptional upregulation of ET-1 (47), whereas in the carotid sinus region, ROS activate an ET-converting enzyme, resulting in increased ET-1 levels (50). In the adrenal medulla, ROS lead to transcriptional upregulation of T-type Ca\(^{2+}\) channels and facilitate Ca\(^{2+}\) influx as well as mobilize Ca\(^{2+}\) stores by activating RyRs via S-glutathionylation (70). These observations suggest that ROS mediate the effects of CIH via affecting multiple cellular targets.

**Evidence for increased ROS in recurrent apnea subjects.** Recent clinical studies documented elevated levels of biomarkers of oxidative stress in recurrent apnea patients (7, 72). Dyugovskaya et al. (14) reported increased ROS generation in CD11c-positive monocytes derived from OSA patients and implicated ROS in the upregulation of adhesion molecules (CD15 and CD11c) and increased adhesion to endothelial cells. These effects are reversed after nasal CPAP treatment. A recent study suggests that antioxidant therapy might be beneficial in restoring vascular function in recurrent apnea subjects. Grebe et al. (19) reported that patients with OSA exhibit reduced vasodilator response, and this effect was prevented by systemic administration of vitamin C, an antioxidant. However, another study reports a lack of oxidative stress in patients with recurrent apneas (40), which could be attributed, in part, to methodological difficulties in assessing ROS levels and gender differences. Nonetheless, the above studies suggest that increased generation of ROS and the resulting oxidative stress is one of the major cellular mechanisms mediating the systemic responses to CIH, including sympatho-adrenal activation.

**MOLECULAR MECHANISMS CONTRIBUTING TO INCREASED ROS GENERATION BY CIH**

The effects of CIH on sympathetic activation, the adrenal medulla, and chemo- and baroreflexes develop over time. The time-dependent effects of a given stimulus are generally attributed to activation of transcriptional mechanisms and the resulting changes in gene expression. Several studies showed that CIH affects a variety of transcription factors, including the hypoxia-inducible factors 1 and 2 (HIF-1 and HIF-2), activator protein 1, NF of activated T cells, and NF-κB [for a review, see Nanduri et al. (42)]. Recent studies suggest that transcriptional activators HIF-1 and HIF-2 are the major molecular mechanisms underlying increased ROS generation by CIH.

HIF-1 is the prototypical member of the HIF family of transcriptional activators and comprises an O\(_2\)-regulated α-subunit and a constitutive β-subunit (74). Hypoxia increases HIF-1 transcriptional activity as a consequence of increased accumulation of HIF-1α protein via decreased O\(_2\)-dependent proline hydroxylation, ubiquitination, and proteasomal degradation (9). HIF-2α (also known as endothelial Per-ARNT-Sim domain protein-1) is another member of the HIF family, which shares ~60% sequence homology to HIF-1α and also interacts with HIF-1β (73).

Continuous hypoxia leads to accumulation of both HIF-1α and HIF-2α (25). In striking contrast, CIH increases HIF-1α and decreases HIF-2α protein in cell cultures as well as in rodents (41). These observations demonstrate strikingly opposite effects of continuous hypoxia and IH on HIF-1 and HIF-2 expression. Yuan et al. (77) reported that a CIH-evoked increase in HIF-1α protein is mediated by a dual mechanism involving activation of mammalian target of rapamycin and S6 kinase pathway and decreased hydroxylation of HIF-1α protein by activation of Ca\(^{2+}\) signaling by ROS. On the other hand, downregulation of HIF-2α by CIH requires activation of Ca\(^{2+}\)-dependent proteases, calpains (41).

Recent studies examined the functional consequences of CIH-induced changes in HIF-1 and HIF-2α. Yuan et al. (76) demonstrated that HIF-1 mediates the transcriptional upregulation of Nox2 by CIH. Hif1α−/− heterozygous mice, exposed to CIH, exhibit a striking absence of 1) elevated plasma catecholamines (an index of sympathetic activation), 2) an augmented chemoreflex, 3) increased ROS generation, and 4) Nox2 upregulation (54, 76). CIH-evoked downregulation of SOD-2 was prevented by overexpressing the transcriptionally active but not the inactive HIF-2α (41). In intact rats, systemic administration of N-acetyl-Leu-Leu-methionyl, a potent inhibitor of calpains, rescues CIH-induced degradation of HIF-2α in the carotid body and adrenal medulla, restores SOD-2 activity, and prevents the increased ROS levels (41). These observations suggest that increased degradation of HIF-2α contributes to a CIH-induced increase in...
ROS via insufficient transcription of antioxidative enzymes, such as SOD-2. Thus the imbalance between HIF-1 and HIF-2 transcriptional activators contributes to a CIH-induced increase in ROS generation via transcriptional regulation of pro- and antioxidant enzymes, and the resulting oxidative stress mediates the effects of CIH on sympathoadrenal activation, as illustrated in Fig. 1.

SUMMARY AND PERSPECTIVE

Although clinical studies have shown that recurrent apnea patients exhibit sympathetic activation, the underlying mechanisms are not known. In recent years, studies using experimental models of IH, the hallmark of apnea, provided important insights into the mechanisms associated with sympathoadrenal activation by chronic IH. There is a general agreement that augmented arterial chemoreflex contributes to the sympathetic activation by CIH. The CIH-induced chemoreflex activation is, in part, due to remodeling of the carotid body chemoreceptor function. In addition to the chemoreflex, evidence is emerging that CIH leads to attenuated baroreflex function, which is, in part, due to reduced carotid baroreceptor activity. Thus an imbalance between the chemoreflex and the baroreflex seems to be a major contributor to sympathetic activation by CIH. Evidence is emerging that CIH leads to reconfiguration of the central neuronal networks associated with sympathetic activation. A major advance in the field of apnea research is the identification of ROS as a major cellular mediator mediating the adverse consequences of CIH on sympathetic function. However, the mechanism(s) underlying ROS generation by CIH, especially in the CNS, need further studies. Studies on cell cultures and rodents led to the identification of HIFs as one of the major molecular mechanisms contributing to sympathoadrenal activation by CIH. Further studies are needed for identifying the role(s) of other transcriptional activators, interactions between the transcriptional activators, and identification of downstream target genes associated with autonomic dysfunction caused by CIH.

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