Sympathoexcitation and arterial hypertension associated with obstructive sleep apnea and cyclic intermittent hypoxia

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Weiss JW, Tamisier R, Liu Y. Sympathoexcitation and arterial hypertension associated with obstructive sleep apnea and cyclic intermittent hypoxia. J Appl Physiol 119: 1449–1454, 2015. First published August 6, 2015; doi:10.1152/japplphysiol.00315.2015.—Obstructive sleep apnea (OSA) is characterized by repetitive episodes of upper airway obstruction during sleep. These obstructive episodes are characterized by cyclic intermittent hypoxia (CIH), by sleep fragmentation, and by hemodynamic instability, and they result in sustained sympathoexcitation and elevated arterial pressure that persist during waking, after restoration of normoxia. Early studies established that 1) CIH, rather than sleep disruption, accounts for the increase in arterial pressure; 2) the increase in arterial pressure is a consequence of the sympathoactivation; and 3) arterial hypertension after CIH exposure requires an intact peripheral chemoreflex. More recently, however, evidence has accumulated that sympathoactivation and hypertension after CIH are also dependent on altered central sympathoregulation. Furthermore, although many molecular pathways are activated in both the carotid chemoreceptor and in the central nervous system by CIH exposure, two specific neuromodulators—endothelin-1 and angiotensin II—appear to play crucial roles in mediating the sympathetic and hemodynamic response to intermittent hypoxia.

chemoreceptor; hypertension; intermittent hypoxia; obstructive sleep apnea; sympathetic nervous system

PREVALENCE OF OBSTRUCTIVE SLEEP APNEA AND ITS RELATIONSHIP TO HYPERTENSION

Early clinical series describing the clinical syndrome of obstructive sleep apnea (OSA) noted an association between upper airway obstructions during sleep and daytime hypertension (35, 44, 55). Although these series were largely uncontrolled for confounding variables such as weight, alcohol intake, and comorbidities, they nevertheless suggested that the nocturnal events had hemodynamic consequences that persisted during waking. The causal relationship between OSA and diurnal hypertension (HTN) was strengthened, however, when mechanistic studies were performed by using two creative animal models that allowed sleep disruption/sleep fragmentation to be uncoupled from nocturnal cyclic intermittent hypoxia (CIH). Brooks and colleagues (7) developed a chronic canine preparation, in which tracheal occlusion mimicked sleep apnea. In this model electroencephalographic (EEG) evidence of sleep triggered closure of a solenoid valve on the animal’s tracheostomy tube. The occlusion was maintained until EEG evidence of arousal appeared, at which time the solenoid was opened restoring airway patency, allowing the animal to return to sleep, thus creating conditions for another occlusion. Like sleep apnea in human patients, the dogs developed repetitive desaturations and sleep fragmentation with daytime sleepiness and arterial hypertension even when awake. Arterial pressure progressively increased until, after 2 mo, the occlusions were halted and daytime hypertension gradually resolved. In an important follow-up study the investigators showed that acoustic sleep disruption, at the same frequency as the obstructive events, produced no increase in pressure, indicating that intermittent hypoxia rather than sleep fragmentation accounted for the daytime hypertension (6). Fletcher and colleagues (20, 21) further emphasized the causal link between CIH and increased arterial pressure. These investigators developed a model of CIH in which rats occupied chambers flushed with nitrogen and room air in alternating fashion. Inspired oxygen concentration thus fluctuated every 30 s between 20.9% and less than 5% for 8 h each day, with the fluctuations induced by alterations in inspired oxygen concentration. This exposure (2, 3), which produces hypocapnic hypoxia rather
than eucapnic or hypercapnic hypoxia as produced by Brooks and coworkers, also resulted in significant increases in arterial pressure.

These animal studies strengthened the physiological connection between CIH and hypertension, but the clinical connection between OSA and hypertension was not firmly established until large prospective epidemiologic investigations such as the Wisconsin Sleep Cohort Study (51, 65) and the Sleep Heart Health Study (45), revealed the substantial prevalence of OSA in the general population and the strong association of OSA with hypertension (30). The Wisconsin Study enrolled Wisconsin state employees to measure the prevalence of sleep apnea, defined as volunteers with positive sleep studies and symptoms of daytime sleepiness. In this population, 4% of working age males met the definition of OSA, with prevalence in females approximately half as great (65, 66). If subjects with positive sleep studies but who denied symptoms were included, the male prevalence swelled to 24% and female prevalence to 9% for an overall prevalence of 16%. These epidemiological studies also further strengthened the link between OSA and systemic elevations in waking arterial pressure. Controlling for all modifiable risk factors for hypertension and baseline pressure, the Wisconsin Study measured relative risk of hypertension as 2.89 in volunteers with moderate or severe sleep apnea at 4-yr follow-up from study entry (51). A follow-up study by the same group confirmed a dose/effect relationship for sleep apnea on hypertension (30) with more severe OSA leading to a greater increase in the relative risk of hypertension. These studies of OSA prevalence and the association of OSA to hypertension suggest that OSA is the most common cause of secondary hypertension.

RELATIONSHIP OF CIH AND OSA TO SUSTAINED SYMPATHOEXCITATION

Many of the early clinical series that suggested a connection between OSA and arterial hypertension further suggested that patients with sleep apnea had high levels of sympathetic activity as assessed by circulating and/or urinary catecholamine levels (18, 24). As noted, many of these studies were uncontrolled, but some did demonstrate reductions in sympathetic transmitter levels after treatment with tracheostomy (24). After establishing that CIH exposure was sufficient to induce increases in arterial pressure, Fletcher and colleagues (23) next used their rat model to provide evidence that CIH-induced increases in arterial pressure involve sustained sympathoactivation. Not only did these investigators establish that pharmacological ablation of sympathetic nerve endings with 6-hydroxydopamine prevented the increase in pressure caused by a 35-day CIH exposure (3), but they further demonstrated that in this model specific regional sympathoexcitation was necessary for increased arterial pressure, as section of the renal nerves prior to the exposure to CIH prevented the pressure rise (23). Sham surgery had no effect, as sham-operated animals had the same increase in arterial pressure after CIH exposure as unoperated animals.

The evidence that exposure to CIH could induce sustained sympathoactivation was later extended to humans by Carlson et al. (8) who by using direct peroneal recordings of muscle sympathetic nerve activity (MSNA) confirmed that OSA patients have increased sympathetic nerve activity compared with nonapneic controls. Waradeker and colleagues (64) then documented that effective treatment of OSA reduces MSNA and reduces arterial pressure, relating the duration patients used nasal continuous positive airway pressure (CPAP) to the magnitude of the decline in MSNA burst frequency and amplitude. Elam and colleagues (19) added to the evidence for OSA induced sympathoactivation, showing with single fiber sympathetic recordings that OSA patients demonstrate increased firing frequency of individual neurons as sympathetic neurons fire with a greater percentage of heartbeats and also fire multiple times with each beat.

Although these patient studies are compelling, patient studies are potentially confounded by the presence of comorbidities and are complicated by variability in disease duration. Human models of CIH exposure have now been used to confirm that in humans, as well as rodents, intermittent hypoxia rather than sleep disruption/sleep fragmentation is the stimulus that leads to sustained sympathoexcitation. Tamisier and colleagues (60) used a commercial altitude tent to expose healthy human volunteers, free of comorbidities, to cyclic hypoxia during sleep. In this paradigm, oscillations in oxygen saturation were induced by intermittently administering supplemental oxygen by nasal cannula. Oxygen saturation fluctuated between ~95 and 85% in these subjects with ~30 oscillations per hour of sleep. Exposures were maintained for 14 (61) or 28 (28) days. Both exposure durations resulted in sustained, significant increases in MSNA whether assessed as increases in sympathetic bursts per minute or as increased bursts per 100 heart beats. Furthermore, these increases in sympathetic outflow accompanied increases in arterial pressure (28, 61) in these healthy subjects. The increases in arterial pressure resolved upon completion of the exposure.

MECHANISMS OF SYMPATHOACTIVATION IN OSA/CIH: EVIDENCE FOR CONTRIBUTIONS FROM THE CAROTID CHEMORECEPTOR AND FROM CENTRAL SITES OF SYMPATHOREGULATION

These studies establish that OSA/CIH hypertension depends on sustained sympathoexcitation, but how the stimulus of cyclic intermittent hypoxia is translated into persistently elevated levels of efferent sympathetic activity remains unclear. In a simplistic way, efferent sympathetic activity is the result of afferent peripheral receptor activity modulating intrinsic activity of central nervous system sites of sympathoregulation. Baroreflex stimulation is sympathoinhibitory, but afferent activity from the peripheral chemoreceptors is sympathoexcitatory. Using their rat model, Fletcher and coworkers (22) were the first to provide evidence that the peripheral chemoreceptor was, indeed, necessary for the development of increased arterial pressure after CIH exposure by denervating the carotid bodies of animals prior to CIH exposure. While sham-operated animals developed increased arterial pressure after 35 days of exposure, animals in which the carotid sinus nerves were surgically interrupted failed to increase pressure. Considerable evidence suggests that exposure to CIH has lasting effects on the carotid body, inducing the specific form of carotid chemoreflex plasticity termed hypoxic acclimatization. Acclimatization refers to the increase in resting ventilation and gain of the ventilatory response to progressive hypoxia that follows exposure to hypoxia of hours or days (17). Characteristically, this enhanced
carotid sinus nerve activity (CSNA) and gain significantly outlast the duration of the hypoxic exposure. Evidence suggests that short-term acclimatization is mediated primarily through changes in the carotid chemoreceptor rather than through central mechanisms of ventilatory regulation (4). Importantly, after acclimatization has occurred, removal of the hypoxic stimulus results in a gradual return of ventilation to baseline; this postexposure persistence of chemoexcitation is termed deacclimatization (53), a change in receptor behavior that might account for symphathoexcitation during waking hours in OSA patients. The hypoxic exposure inducing acclimatization need not be continuous (9, 27). For example, Peng and colleagues (47-49) reported an increase in CSNA during both normoxia and rechallenge to hypoxia in rats exposed for 14 days to cyclic hypoxia (20 s every 5 min, 8 h/day). Supporting the hypothesis that untreated OSA may increase chemosensitivity is a report that patients treated with nasal CPAP demonstrate a decrease in the ventilatory response to hypoxia relative to their untreated baseline (63).

These studies indicate that the carotid chemoreceptor contributes to OSA/CIH-induced symphathoexcitation, but how CIH exposure is translated into enhanced peripheral chemosensitivity is unclear. Although there are many possible candidates to explain acclimatization (4), considerable evidence implicates the neuromodulators endothelin-1 (ET-1) and angiotensin II (AT II) as major contributors. Data now support a critical role for endothelin as a mediator of augmented chemosensitivity in acclimatization after both continuous hypoxia (12, 13) and after CIH (57, 58). Endothelin is a 21-amino acid peptide found in endothelium, in the central nervous system, and in Type 1 cells (glomus cells) in the carotid bodies (43). There has been controversy as to whether ET-1 alters CSNA primarily by altering blood flow or by direct action on the chemosensory cells, but isolated recordings have firmly established that ET-1 has direct effects on glomus cells (11). Two weeks of continuous hypoxia has been shown to increase expression of the endothelin A (ETA) receptor, the endothelin B receptor (ETB), and preproendothelin, the precursor of endothelin, in the carotid body (12). Furthermore, analysis of chemoreceptor activity by using carotid sinus nerve recordings and a selective ETA antagonist suggested that the increases in chemoreceptor activity paralleled the increases in ET-1 and ETA expression. Recent evidence also suggests a role for ET-1 in enhanced chemosensitivity after CIH. Rey and colleagues (56) examined ET-1 immunoreactivity in cats exposed to CIH 8 h/day for 4 days. They observed an increase in isolated carotid body response to exogenous ET-1 after CIH and a reduction in activity after treatment with bosentan, a mixed ETA and ETB blocker. Recently, Pawar and colleagues (46) showed that in neonatal rat pups exposed to CIH for 10 days there was an enhanced basal level of ET-1 release in the carotid body, the carotid body response to exogenous ET-1 was enhanced, and ETA receptor message was increased but not ETB message. Significantly, malondialdehyde (MDA), an index of ROS levels, was elevated in the carotid bodies of pups exposed to CIH. Systemic administration of manganese tetakis (1-methyl-4-pyridyl) porphyrin pentachloride, a scavenger of $O_2^-$, prevented upregulation of ETA, ROS activity, and the augmented hypoxic response.

Although the role of endothelin in modulating peripheral chemosensitivity (and thus sympathetic activity) has been best studied, a number of studies also implicate activity of the renin-angiotensin-aldosterone system as a local modulator of chemoreceptor sensitivity. Fung and coworkers (26, 33) have identified components of renin-angiotensin signaling in the carotid body of rats, and expression of these peptides is enhanced after exposure to CIH. Marcus and colleagues (39) also demonstrated that CIH exposure of rats to CIH for 28 days enhances carotid expression of both the angiotensin II type-1 receptor (AGT1R) and, furthermore, elevates carotid production of both superoxide and of the gp91(phox) component of NADPH oxidase, a signaling constituent downstream of angiotensin II binding to its receptor.

Although physiological studies suggest the carotid chemoreceptor is necessary for the development of CIH-induced hypertension, recent studies examining central sites of symphathoexcitation suggest enhanced chemosensitivity is not sufficient to account for the increase in arterial pressure after CIH exposure. Knight and coworkers (32) showed that intracerebroventricular (ICV) infusion of losartan, an AT1R blocker, in rats exposed to CIH for 7 days prevented the increase in arterial pressure displayed by vehicle-infused control animals. Da Silva (16) also infused losartan, and blockers of other angiotensin receptors, bilaterally into the hypothalamic paraventricular nuclei in CIH-exposed animals. In this study blockade of angiotensin 1–7 receptor, angiotensin II type-2 receptor, and AGT1R all prevented the increase in arterial pressure demonstrated by control animals. Interestingly, the GABA (A) receptor agonist mucimol also reversed the increase in pressure after CIH. Saxena and coworkers (59) recently used Adeno-associated virus and small hairpin RNA against AT1a receptor to knock down expression of the receptor in the subfornical organ (SFO). Injection of a scrambled control sequence was used for a sham intervention. Only the animals injected with the control demonstrated an increase in arterial pressure after the 7 days of CIH exposure, and only sham-injected animals had increased staining for FosB/deltaFos B staining in the median preoptic nucleus and in the paraventricular nuclei. Finally, in a study that investigates the endothelin system also contributes to central sympatohexic action after CIH exposure, Huang and colleagues (31) showed that 3-wk CIH exposure increases expression of the endothelin type A (ETA) receptor in the subfornical organ of rats, and CIH-exposed rats had an enhanced sympathetic and arterial pressure response to ICV ET-1 relative to sham-exposed animals.

Although the signaling pathways activated in glomus cells and CNS by ET-1 and renin-angiotensin-aldosterone are unknown, data have established that one consequence of ET-1 activation of ETA receptors in the carotid body appears to be activation of the glutamate NMDA receptors present on glomus cells (37). CIH exposure also increases expression of NMDA NR1 receptors in carotid body. Furthermore, ET-1 also activates glutamate AMPA receptors in carotid body, and CIH exposure increases expression of the AMPA-type GluR1 receptors. Based on these findings, one hypothesis is that ET-1 induces a form of plasticity in the chemoreceptor that is mediated through glutamate receptors. This hypothesis is strengthened by the findings of Chan and coworkers (10) that sympathoexcitation induced when angiotensin is injected into the rostral ventral lateral medulla requires glutamatergic signaling. In carotid body, evidence suggests that both NMDA
and AMPA receptors mediate endothelin’s influence on CSNA, consistent with a form of glutamatergic plasticity (37).

HUMAN STUDIES SUGGESTING A ROLE FOR RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM INVOLVEMENT IN CIH/OSA SYMPATHOACTIVATION AND HYPERTENSION

Human studies have not yet confirmed ET-1 or RAS signaling in the human chemoreflex or central nervous system response to CIH, but studies do support a role for RAS in OSA/CIH hypertension. A randomized crossover trial indicates that the angiotensin Type I receptor (AGTR1) blocker valsartan reduces mean arterial pressure (MAP) more effectively than therapy with nasal CPAP in a group of hypertensive OSA patients (50). While this may be a nonspecific antihypertensive effect, it suggests a role for RAS in OSA hypertension that warrants further investigation. Studies in normal volunteers further suggest a role for RAS in the response to CIH. In a group of normal volunteers exposed to CIH for 6 h, Foster and colleagues (25) used a double-blind, placebo-controlled, randomized crossover design to show that the AGTR1 blocker losartan prevented the increase in arterial pressure evident with placebo. Although this model has questionable parallels to OSA since there was no increase in peripheral chemosensitivity, the results are intriguing, particularly because the same investigators demonstrated that losartan also abolished evidence of oxidative stress using the same paradigm (52). Our own (unpublished) preliminary studies found that human carotid body glomus cell tumor tissue expresses components of glutamatergic signaling which in other studies is activated in nervous tissue by Ang II and ET1, raising the possibility that they may modulate chemoreflex sensitivity in humans as well as animals.

FUTURE DIRECTIONS AND LESSONS FROM OTHER MODELS OF SYMPATHOACTIVATION

The literature cited indicates that exposure to CIH, either in model systems or in the clinical syndrome of OSA, leads to sustained sympathoactivation. Furthermore, this sympathoactivation appears to result from both enhanced peripheral chemosensitivity and from altered central sympathoregulation. Finally, enhanced activity of both the endothelin system and the renin-angiotensin system contribute to the altered sympathoregulation in both carotid body and in the central nervous system. But many questions remain.

What is the relationship between altered chemosensitivity and altered central sympathoregulation after CIH exposure? Denervation of the carotid chemoreceptor prevents CIH hypertension (22). Similarly, blockade of AGTR1 receptors in the subfornical organ prevents CIH-induced hypertension (16, 32). Are the changes in peripheral chemosensitivity necessary for the changes in central sympathetic processing? Are changes in both carotid body and central sites of sympathoregulation due to enhanced circulating levels of ET-1 and AT II? Or does intermittent hypoxia directly stimulate increased expression of ET-1 and Ang II signaling components? One of the more provocative recent findings was the report of Abdala and coworkers (1) who found that carotid body denervation of juvenile animals prevented the expected increase in arterial pressure in spontaneously hypertensive rats. These animals are not believed to experience CIH, but this finding suggests that enhanced peripheral chemosensitivity contributes to sustained sympathoexcitation, nevertheless. There are no reports to date of altered carotid body or SFO expression of ET-1 or Ang II signaling in these rats.

Are ET-1 and Ang II signaling both necessary in CIH hypertension? Not only are expression of both endothelin and angiotensin signaling constituents enhanced in carotid body and in CNS after CIH exposure, but ICV/SFO injection of either an ETA blocker or an AGT1R blocker alters the increase in arterial pressure after CIH (16, 31). This suggests the possibility that ET-1 and AT II act in series or that they act through the same pathway.

Why does the sympathetic/hemodynamic response to CIH/OSA vary among individuals? While the studies cited indicate that exposure to CIH/OSA is associated with a significant increase in arterial pressure, the mean responses mask significant variability among subjects and patients, variability that does not seem to be accounted for solely by differences in exposure or disease severity. This variability has not been examined in a systematic way but raises intriguing questions about susceptibility that might relate to genetic or epigenetic differences in sympathetic, RAS, or endothelin control.

Does CIH exposure produce an inflammatory response similar to that demonstrated in other forms of “stress hypertension”? A series of observations published over the last decade have changed our concept of neurogenic HTN. Among these observations are the following: 1) a number of humoral [e.g., angiotensin II (54)] and environmental [e.g., particulate air pollution (14, 15), stress (34), salt intake (5)] exposures are associated with hypertension and high sympathetic activity; 2) clinical and animal studies indicate HTN is associated with increased circulating markers of inflammation (38); 3) bidirectional communication occurs between central sites of sympathoregulation and the innate and adaptive immune systems such that sympathetic outflow increases generation of proinflammatory cytokines that act centrally to enhance sympathetic outflow; and 4) angiotensin II (29, 40) is generated in the kidney but also produced locally in carotid chemoreceptor (36), central sites of sympathoregulation (62, 67), and in T-helper lymphocytes (41, 42)—Ang II thus acts directly to enhance sympathetic outflow but also acts indirectly within T-helper (Th) cells to produce inflammation which further enhances sympathetic activity (29, 42). We performed reverse transcription PCR for angiotensin II, the angiotensin 1 receptor (AT1), and angiotensin converting enzyme 1 (ACE) in CD4+ cells from normal human volunteers. We used human umbilical vein endothelial cells (HUVEC) as a positive control. The size of the ACE PCR product from CD4+ cells was larger than the expected 408 bp, indicating alternative splicing. GADPH was used as a housekeeping gene, and PCR product was not detected when reverse transcriptase was omitted from the reaction. These studies confirmed that human Th cells, like murine Th cells (42), contain the components of the RAS and are suitable for assay of gene expression changes after CIH exposure. This may be a useful way in human volunteers or OSA patients to assess changes in RAS expression in response to CIH exposure.

Based on the evidence detailed, we propose the following paradigm for the effect of CIH sympathoexcitation and hypertension. We speculate that CIH exposure in vulnerable individuals increases renin-angiotensin and endothelin activity in

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crucial organs: CNS, carotid body, kidney, and Th cells. Activation of these pathways leads to sympathoactivation in OSA patients and in human volunteers and animals exposed to CIH. CIH increases oxidative stress directly, and through RAS/ET-1 activation, which in turn amplifies sympathoactivation. Sympathoactivation and inflammation then lead to hypertension. Genetic and/or epigenetic predisposition or individual differences in genomic and/or epigenetic responses to OSA/CIH may explain why some individuals are particularly vulnerable to this cascade of events.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

J.W.W., R.T., and Y.L. conception and design of research; J.W.W., R.T., and Y.L. analyzed data; J.W.W., R.T., and Y.L. interpreted results of experiments; J.W.W. drafted manuscript; J.W.W. edited and revised manuscript; J.W.W., R.T., and Y.L. approved final version of manuscript; R.T. and Y.L. performed experiments.

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