Medullary Respiratory Network Drives Sympathetic Overactivity and Hypertension in Rats Submitted to Chronic Intermittent Hypoxia

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Hypertension is a pathological condition affecting up to one third of adult population worldwide.\(^1\) Over the last few decades, the remarkable progress of the pharmacological antihypertensive therapies, such as \(\beta\) adrenergic blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor type 1 antagonists, and diuretics, combined with physical activities and dietary orientations, contributed significantly to improve life quality of millions of hypertensive patients around the world by lowering their arterial pressure. However, in \(\approx 30\%\) of hypertensive patients the arterial blood pressure remains elevated in spite of the use of different pharmacological strategies,\(^1,4\) indicating not only that these therapies are not effective for all patients but also that the mechanisms underpinning the chronic increase of arterial pressure are not completely understood. In this context, a relevant example is the hypertension observed in patients suffering of the syndrome of obstructive sleep apnea (OSA). Over the last 2 decades, it has been demonstrated that the OSA, a condition that affects \(\approx 20\%\) of adult population in the United States,\(^5\) is an important risk factor for the development of arterial hypertension,\(^4\) and it is one of the major causes of secondary hypertension in patients presenting hypertension resistant to pharmacological treatment.\(^7\) Regardless of the marked correlation between OSA and arterial hypertension,\(^8\) the mechanisms contributing to the development of hypertension in OSA patients are not fully elucidated.

There is evidence that hypertension in OSA patients is markedly associated with the long-term exposure to episodic hypoxemia (or intermittent hypoxia) as a consequence of recurrent obstructions of upper airways during sleep.\(^9\) The critical role of chronic intermittent hypoxia (CIH) exposure in the development of cardiovascular changes induced by OSA is supported not only by clinical studies reporting the beneficial cardiovascular effects of the treatment of OSA patients with continuous positive airway pressure\(^10-13\) but also by experimental data showing that animals exposed to CIH develop sustained hypertension,\(^14\) an effect prevented by previous denervation of peripheral chemoreceptors.\(^15\) In this brief review, we discuss recent data about the possible mechanisms underpinning the hypertension induced by CIH exposure, with focus on the development of the sympathetic overactivity that is associated with this pathophysiological condition. Here, we present a novel concept in the scenario of neurogenic hypertension with regard to the coupling between respiratory and sympathetic activities. The changes in this coupling should be considered as an important component for the development of augmented sympathetic activity in CIH as well as in other experimental models of sympathetic-mediated hypertension. We speculate that this new concept can be translated to some clinical aspects of essential hypertension and that it may provide support for new strategies for the control of the arterial pressure.

Sympathetic Overactivity: The Main Mechanism Underpinning Arterial Hypertension Elicited by CIH

Distinct paradigms of experimental CIH are found in the literature, such as different species used (mainly rats and mice), time of exposure (from hours to months), hypoxia level, and duration of each episode.\(^16-20\) In spite of these differences, there is a consensus that CIH exposure leads to sustained arterial hypertension.\(^21,22\) Previous studies documented that CIH rats exhibited dysfunctions in vascular reactivity, including reduced nitric oxide-dependent vasodilatation\(^23\) and enhanced vasoconstrictor response to endothelin-1,\(^24\) supporting the concept that an impairment of vascular function contributes to the maintenance of hypertension observed after CIH exposure.\(^25,26\) On the other hand, the pioneering studies by Fletcher et al\(^14\) showed that either the chemical sympathetic denervation or the renal sympathetic denervation\(^27\) prevented the development of hypertension in CIH rats, suggesting the critical role for the sympathetic nervous system in the development of hypertension in this experimental model. Moreover, we reported that CIH rats exhibited larger depressor responses to ganglionic blockade, higher levels of basal noradrenaline, and enhanced sympathetic-mediated systolic blood pressure variability,\(^19,20\) indicating that the
sympathetic vasomotor tonus of CIH rats is significantly enhanced. Indeed, by direct recordings of sympathetic vasoconstrictor nerves, we also verified that CIH rats exhibited higher baseline and chemoreflex-induced sympathetic activities, providing additional support to the concept that CIH enhances the sympathetic outflow to the heart and blood vessels. These experimental findings are consistent with clinical data showing that healthy subjects exposed to intermittent hypoxia exhibited higher levels of basal muscle sympathetic activity and higher sympathetic chemoreflex response. However, it remains unclear how CIH interferes with the modulation of the sympathetic outflow, producing, as a consequence, a sympathetic overactivity and hypertension.

**Mechanisms Involved in the Generation of Sympathetic Overactivity in Response to CIH**

An important question to be answered is why the sympathetic activity is increased in response to CIH. Studies by Prabhakar and Kumar suggested that impairment of the baroreflex inhibitory control of sympathetic nervous system is an important mechanism contributing to the development of sympathetic overactivity and hypertension in animals submitted to CIH. However, the observed increase in arterial pressure induced by CIH seems to precede the baroreflex dysfunction. Studies from our laboratory performed in awake rats submitted to CIH during 10 days demonstrated that the development of arterial hypertension is not secondary to a reduction in the gain of baroreflex, which, in fact, was found to be increased in these animals. Therefore, these data from our laboratory indicate that sympathetic overactivity and hypertension in CIH rats are not related to an impairment of the baroreflex function.

With respect to the peripheral chemoreflex, there is evidence indicating that this reflex is exaggerated after CIH. The activation of peripheral chemoreceptors in rats produces reflex sympathoexcitation associated with an enhanced respiratory drive. In CIH rats, it was observed that sympathetic and tachypneic chemoreflex responses are enhanced, indicating that a facilitatory effect of CIH may occur in the glomus cells located in the carotid bodies, in the synaptic processing of this reflex at the brain stem nuclei, or both. At the peripheral level, it was demonstrated that CIH produces a sensitization of chemosensitive glomus cells by mechanisms dependent on the generation of reactive oxygen species and changes in angiotensin II transmission. At the central nervous system level, important alterations in mechanisms of neurotransmission in neuronal groups critically involved with the processing of sympathetic chemoreflex response, such as in the nucleus tractus solitarius, the hypothalamus, and the ventral medulla, have been reported. These peripheral and central alterations of chemoreflex pathways induced by CIH contribute to the facilitation of sympathetic chemoreflex response, and it represents an important mechanism of long-lasting excitation of medullary sympathetic neurons in response to new episodes of hypoxia and sustained increase of baseline sympathetic outflow after CIH.

In addition to changes in the synaptic transmission in brain stem nuclei involved with the control of sympathetic activity, the exaggerated sympathetic chemoreflex response of CIH rats may be consequent to the enhancement of the respiratory reflex response. This hypothesis is based on the fact that processing of sympathoexcitatory response of chemoreflex is, at least in part, dependent on the activation of respiratory brain stem network because of interactions between respiratory and sympathetic neurons. More importantly, not only the sympathetic reflex response to hypoxia is markedly modulated by respiratory activity but also baseline sympathetic discharge exhibited rhythmic oscillations entrained with the respiratory cycle (Figure 1), even in the absence of peripheral pulmonary and baroreceptors feedback control. These observations clearly indicate the existence of a central coupling between respiratory network and sympathetic nervous system. Considering that the mechanisms generating high levels of sympathetic activity and arterial hypertension in CIH rats are complex and involve several mechanisms, it is reasonable to take into consideration that alterations in the functioning of central respiratory network may impose an additional excitatory drive to the presympathetic neurons. This hypothesis is supported by the fact that CIH produce substantial changes in the mechanisms controlling baseline respiratory activity. Therefore, in our view, changes in the mechanisms of interaction between respiratory and sympathetic networks should be considered as a novel mechanism contributing to the development of sympathetic overactivity and hypertension in CIH rats.

**Central Respiratory–Sympathetic Coupling: From a Physiological Concept to a Novel Mechanism Underpinning Sympathetic Overactivity Induced by CIH**

The first evidence of possible connections between neurons involved in the regulation of respiratory and autonomic nervous system was described by Traube and Hering. Traube observed the occurrence of large blood pressure waves after stopping the artificial ventilation in vagotomized and paralyzed dogs and cats, whereas Hering noticed that each of these arterial pressure waves, later named Traube–Hering waves, was correlated with a movement of the respiratory muscles (Figure 1). Similarly on animals with intact vagus nerves, Traube also observed an increase of heart rate during inspiration (Figure 1), which was associated with a decrease in the vagal activity to the heart, a phenomenon that was also experimentally documented. Therefore, Traube was the first to suggest that the sympathetic and parasympathetic innervations to the cardiovascular system are significantly influenced by the respiratory activity. Subsequent studies by Adrian et al confirmed, by direct recordings of sympathetic nerves, that the sympathetic activity, in fact, exhibits rhythmic oscillations associated with the respiratory activity. After these classic studies, several important studies explored the mechanisms of respiratory–sympathetic coupling in different species as well as in specific sympathetic branches. The physiological role of respiratory fluctuations on the sympathetic and parasympathetic activities to the cardiovascular system in mammals is still not clearly defined. However, it has been suggested that coordination of
autonomic and respiratory activities may improve the blood delivery to lungs,\textsuperscript{66} optimizing the blood gas exchange and tissues perfusion, especially in conditions of metabolic challenges, such as hypoxia.\textsuperscript{63} At the level of single neurons, it is evident that the synchrony of respiratory and sympathetic systems has potential functional implications, because it may contribute to the summation of neuronal inputs to pre-sympathetic neurons and consequently increase the efficacy of neuroeffector transmission at specific periods of respiratory cycle.\textsuperscript{64}

The respiratory–sympathetic coupling has been suggested to be primarily generated by interactions between medullary central respiratory generator and presympathetic neurons (Figure 1).\textsuperscript{52–55} In fact, important nuclei of brain stem controlling the respiratory activity are anatomically intermingled with neurons involved in the generation and regulation of sympathetic activity.\textsuperscript{22} However, direct recordings of the neuronal activity revealed that presympathetic neurons located in the rostral ventrolateral medulla (RVLM) exhibit distinct patterns of activity entrained with the respiratory cycle.\textsuperscript{54} Thereby, it has been proposed that the ventral surface of medulla is one of the most important areas in the brain stem responsible for integration of respiratory and sympathetic neuronal activities.\textsuperscript{22,55,65,66} The inspiratory and expiratory brain stem neurons, especially those from ventral respiratory column, the pons, or the retrotrapezoid nucleus,\textsuperscript{67–69} may establish interactions with the RVLM neurons and drive excitatory or inhibitory inputs.\textsuperscript{69} These connections, acting directly or indirectly via projections to neurons located in the caudal ventrolateral medulla,\textsuperscript{70} provide excitatory and inhibitory inputs for the generation of respiratory rhythmicity of sympathetic activity (Figure 1). For visceral vasoconstrictor nerves, which control vascular smooth muscle tonus, the respiratory–sympathetic coupling pattern consists of an excitatory modulation of sympathetic activity during inspiratory phase, with a peak of activity during the late inspiration or postinspiration\textsuperscript{22,69} (Figure 1).

More than an important and beneficial physiological role, maintained alterations or overactivation of mechanisms involved with respiratory–sympathetic coupling may have pathological implications to the cardiovascular system. This concept was originally suggested by Cushing,\textsuperscript{71} who demonstrated in patients after traumatic head injury that elevated intracranial pressure produced severe hypertension accompanied by an increase in the magnitude of Traube–Hering waves. Indeed, there is evidence that abnormal respiratory–sympathetic coupling plays a significant role in the development of hypertension. The first experimental evidence of an altered respiratory–sympathetic coupling associated with hypertension was demonstrated by Czyzyk-Krzeska and Trzebski,\textsuperscript{72} who reported that the respiratory-related peak of sympathetic activity of anesthetized, vagotomized adult spontaneously hypertensive rats was shifted from expiratory to inspiratory phase. More recently Simms et al\textsuperscript{73} provided clear evidence that the amplitude of respiratory bursts of sympathetic activity is amplified and drives larger Traube–Hering waves in juvenile spontaneously hypertensive rats before the development of hypertension. These findings support the concept of a direct causal relationship between the augmented respiratory–sympathetic coupling and increased vascular resistance in spontaneously hypertensive rats.

Based on these pathophysiological aspects of the mechanisms of respiratory–sympathetic coupling, we evaluated the possibility that changes in the strength of interactions between respiratory and sympathetic neurons may contribute to increase baseline sympathetic activity in CIH rats. We initially examined this possibility using the decerebrated artificially perfused in situ working-heart brain stem preparations of rats,\textsuperscript{74} in which we characterized the firing pattern of thoracic sympathetic nerves of CIH rats along the respiratory...
cycle. In the working-heart brain stem preparations, the brain stem circuitry controlling respiratory and sympathetic activities are maintained intact and generate a pattern of sympathetic discharge that resembles the pattern recorded in vivo, with the sympathetic activity exhibiting a phasic increase during inspiration and a peak during late inspiration/postinspiration (Figure 2). This pattern of activity suggests that in every respiratory cycle, inspiratory and postinspiratory neurons predominantly modulate the activity of RVLM presympathetic neurons. In working-heart brain stem preparations of rats previously submitted to CIH, sympathetic activity also presented peaks of discharge during the late-inspiratory/postinspiratory phases. However, CIH rats exhibited additional bursts of sympathetic activity during the late-expiratory phase (late-E, before the phrenic bursts), inducing an increase in sympathetic activity during the expiratory phase (Figure 2). Interestingly, these alterations of respiratory–sympathetic pattern of CIH rats were tightly associated with marked changes in baseline respiratory pattern. By direct recordings of the abdominal motor nerve activity, we found that CIH rats exhibited an enhanced and novel peak of activity in abdominal motor nerves during late-E, which was not seen in control rats (Figure 2).

These findings indicate that, at baseline conditions, CIH rats present a pattern of enhanced/forced expiration whereas in control rats the expiration is essentially passive (low amplitude of abdominal activity, Figure 2). It is important to note that the emergence of late-E bursts in the activity of both abdominal and sympathetic nerves in CIH rats was significantly correlated, and the reduction of respiratory drive selectively attenuated the CIH-induced respiratory activity of sympathetic nerve, suggesting a causal relationship between active expiration and sympathetic overactivity in this experimental model. In addition, CIH working-heart brain stem preparations exhibited larger Traube–Hering waves (Figure 2), indicating that this pattern of respiratory–sympathetic coupling seen in CIH is transmitted to the vessels and increases the vascular resistance. Altogether, these data strongly support the concept that RVLM presympathetic neurons of CIH rats receive an additional excitatory modulation during the expiratory period, a phenomenon that may reveal a novel and critical mechanism for the development of hypertension in this experimental model.

In awake freely moving rats, we also evidenced that the high levels of arterial pressure of CIH rats exhibited a marked increase in the power of the high-frequency variability (related to Traube–Hering waves) of systolic arterial pressure. This high-frequency component of arterial pressure (in rats, 0.75–3.00 Hz) is associated with the respiratory modulation on the arterial pressure and may be associated with the following: (1) changes in intrathoracic pressure during the respiratory cycle, which increase the venous return and the cardiac output, stimulating the arterial baroreceptors; (2) signals arising from the lungs, especially from stretch receptors, which, in turn, reflexly modulate sympathetic activity; and (3) alterations in the central coupling between respiratory rhythmogenic and sympathetic neurons in the brain stem. The latter aspect supports our original hypothesis obtained in in situ preparations and validates the idea that changes in central respiratory–sympathetic coupling contribute in a very important manner to the augmented sympathetic outflow in CIH rats. Therefore, our data indicate that CIH enhances baseline expiratory activity, which, in turn, increases the sympathetic activity as a consequence of a strengthened central coupling between the expiratory and presympathetic neurons of the brain stem.

Changes in the Properties of Respiratory Neurons in the Ventral Medulla

The alterations observed in baseline expiratory activity of CIH rats may involve changes in the activity of expiratory neurons located in the ventral respiratory column, including those located in Bötzinger complex (BötC), which is considered a major source of expiratory neurons. The BötC contains mainly 2 types of expiratory neurons, postinspiratory neurons, neurons that exhibit a rapid depolarization after the end of inspiration and a decrementing pattern of activity during the expiratory phase, and augmenting expiratory neurons, neurons with a augmenting pattern of activity during the expiratory phase, reaching a peak during late-E. We suggest that the emergence of late-E activity in abdominal nerve of CIH rats at resting conditions may be associated with a higher excitatory drive to augmenting expiratory neurons of BötC, which, in turn, may send excitatory inputs to presympathetic neurons of RVLM (Figure 2). Another possible mechanism is based on recent studies showing that the retrotrapezoid nucleus/parafacial respiratory region, a ventromedullary region located rostral to the RVLM and ventral to the facial nucleus, also contains expiratory neurons that are critical for the generation of enhanced expiratory activity. These retrotrapezoid nucleus/parafacial respiratory region neurons are thought to interact not only with the BötC expiratory neurons but also with the presympathetic neurons of RVLM. In this scenario, the expiratory neurons of retrotrapezoid nucleus/parafacial respiratory region may also be considered as a potential target of plasticity, leading to coupled expiratory and sympathetic overactivation observed in rats submitted to CIH (Figure 2). Therefore, it is plausible that the expiratory neurons of BötC or retrotrapezoid nucleus/parafacial respiratory region are the major source of phasic excitation to the sympathetic activity during late-E phase, potentially by direct interactions with the RVLM presympathetic neurons, because there is no evidence that caudal ventrolateral medulla neurons display late-E activity either at baseline or during hypoxic conditions. However, it is safe to quote that these proposed connections are not yet proven and are currently under investigation in our laboratory.

Final Remarks and Perspectives

In this brief review, we presented a novel concept that changes in the coupling of respiratory and sympathetic activities contribute to increase sympathetic outflow and arterial pressure in rats submitted to CIH. However, to explain in detail how the changes in the respiratory network produce additional excitatory inputs into the sympathetic nervous system, we still need to identify the possible electrophysiological changes in the different subgroups of sympathetic and respiratory neurons of the ventral medulla of CIH rats and determine...
how these possible changes are translated in sympathetic overactivation and hypertension. Because the changes in the respiratory–sympathetic coupling are also associated with high sympathetic levels in other experimental models of hypertension, including spontaneously hypertensive rats and angiotensin II-salt-dependent hypertension, we speculate that this phenomenon is not restricted just to 1 experimental model of hypertension and highlights the respiratory–sympathetic coupling as a novel and relevant component of enhanced baseline sympathetic activity, which may be present in patients with neurogenic hypertension. These experimental observations open new and interesting perspectives about the clinical evaluation of the pattern of respiratory activity in hypertensive patients, to verify whether this phenomenon described in the CIH experimental models is also exhibited by humans. In an affirmative case, we may predict that a new line of clinical investigation will be available for a better understanding of the mechanisms underlying essential hypertension in humans, especially on those who are resistant to the conventional antihypertensive pharmacological treatment, with implications for the development of voluntary respiratory maneuvers for the control the sympathetic outflow and consequently the high blood pressure.

Acknowledgment

We thank Dr Carlos Eduardo L. Almado for his important contribution to the final form of this brief review.

Sources of Funding

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2009/50113-0), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC 29.495/2010–3).

Disclosures

None.

References


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Hypertension. 2012;60:1374-1380; originally published online October 29, 2012;
doi: 10.1161/HYPERTENSIONAHA.111.189332

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