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# Hypertension in sleep apnea: The role of the sympathetic pathway

Chronic intermittent hypoxia, a phenomenon that occurs during episodes of sleep apnea, has been shown to produce hypertension independent of obesity, diabetes, and other potentially confounding factors in patients with sleep apnea.

This article discusses the research of our laboratory and collaborating researchers in elucidating the effects of chronic intermittent hypoxia and the mechanisms responsible for the hypertension induced by a reduction in partial pressure of oxygen ($P_{O_2}$). Using Sprague-Dawley rats as a model, we have focused on the chemoreflex pathway, which arises from the carotid body and is the primary sensor of reduced arterial oxygen levels.

## RELATIONSHIP BETWEEN $P_{O_2}$ AND HYPERTENSION

Reduced $P_{O_2}$ could theoretically cause hypertension by a direct effect on smooth muscle, or hypertension could be mediated by endocrine factors or through neural pathways.

Considerable evidence points to the sympathetic neural pathway:

- Some patients with chronic sleep apnea have increased basal sympathetic activity, which can be measured from a nerve containing sympathetic fibers entering a muscle.[1–3]
- Rats exposed to chronic intermittent hypoxia have an exaggerated sympathetic response to acute hypoxia compared with controls, as measured in cervical, renal, and muscle nerves.[4]
- Blocking sympathetic nerve activity in rats using 6-hydroxydopamine prevents chronic intermittent hypoxia-induced hypertension from developing.[5]

## THE CHEMOREFLEX PATHWAY: AN IDEAL MODEL

What is the source of increase in sympathetic activity, and why is it not modulated by baroreceptors? To answer these questions, the chemoreflex pathway **(Figure 1)** may be a good model. It consists of:

- Carotid body chemoreceptors that lie in the bifurcation of the common carotid artery. They respond to decreased arterial $P_{O_2}$ by releasing transmitters to activate sensory nerve fibers within the carotid body.
- The carotid sinus nerve, which joins the glossopharyngeal nerve and carries the sensory afferent fibers that are activated by the transmitters. These nerves have their cell bodies within the petrosal ganglia. Their axons project into the brainstem.
- The afferent nerve fibers that enter the nucleus of the solitary tract, where they make synaptic connections with neurons that relay information to the ventral medulla and onward through the intermediolateral columns of the spinal cord and out through the sympathetic ganglia.
- The adrenal glands, blood vessels, and the heart, to which information is ultimately transferred.

The chemoreflex pathway turns out to be an ideal place to look for changes that occur during intermittent hypoxia, for several reasons: the carotid body is a primary sensor for reduced arterial $P_{O_2}$, electrically stimulating the carotid body pathway increases arterial pressure and heart rate, and severing the nerve in rats has been shown to prevent the hypertension of chronic intermittent hypoxia.[6]

## THE HYPOTHESIS: CHEMOREFLEX PATHWAY ACTIVATION LEADS TO SUSTAINED ELEVATION IN ARTERIAL PRESSURE

Our working hypothesis is that increased activation of the carotid body chemoreflex pathway in chronic intermittent hypoxia leads to sustained elevation in arterial pressure. Our research has focused on learning why this occurs.

### The protocol

Our protocol, developed by Prabhakar and colleagues,[7] is as follows: Sprague-Dawley rats are exposed to chronic intermittent hypoxia (15 seconds of 5% $O_2$,

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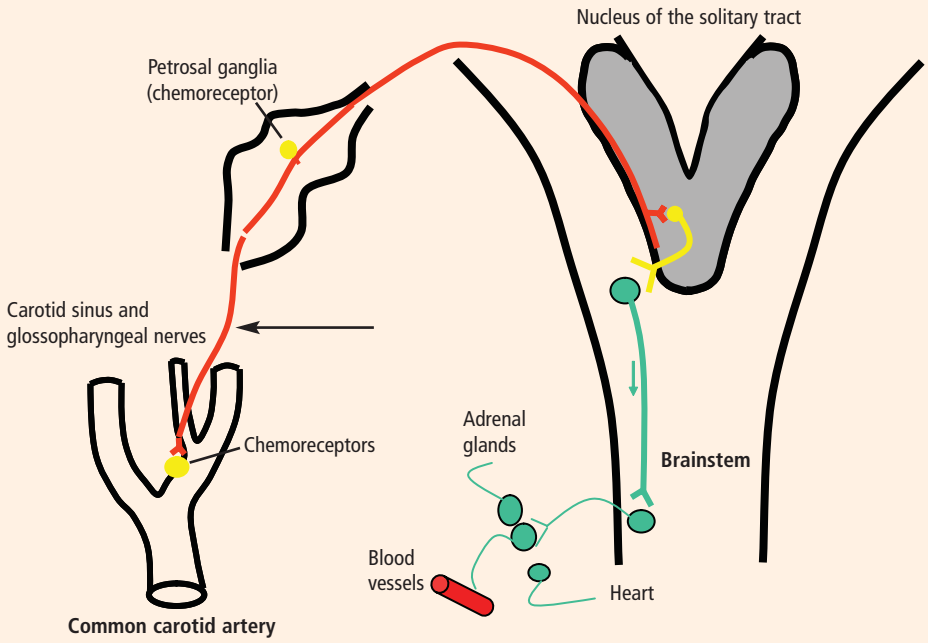


**FIGURE 1.** The chemoreflex pathway. Carotid body chemoreceptors respond to decreased arterial $Po_2$ by releasing transmitters to activate sensory nerve fibers within the carotid body. The carotid sinus and glossopharyngeal nerves, whose cell bodies reside in the petrosal ganglia, transfer the sensory afferent fibers to the brainstem via the nucleus of the solitary tract. There the afferent nerve fibers make synaptic connections with neurons, which relay information through the spinal cord and sympathetic ganglia to the adrenal glands, blood vessels, and heart.

followed by 5 minutes of 21% $O_2$ for 8 hours per day). After 10 days, arterial pressure elevations in the range of 10 to 25 mm Hg are demonstrated[7,8] (other laboratories with different protocols achieve different degrees of blood pressure elevation[6,9]).

### Intermittent hypoxia increases carotid body activity

Using this protocol, Prabhakar and colleagues studied carotid body discharge.[7] The carotid body glomus cells respond to $Po_2$ via nerves that innervate the region. After 10 days of exposure to chronic intermittent hypoxia, the baseline activity in the carotid body was higher than normal and the response to acute hypoxic stimuli was exaggerated.

From this it was concluded that activity from the carotid body was increased from chronic intermittent hypoxia, leading to increased arterial pressure. We would expect baroreceptors to buffer this response, but they do not appear to do so.

### Intermittent hypoxia inhibits activity in the nucleus of the solitary tract

To explore whether other sites along the chemoreflex pathway were also susceptible to reduced $Po_2$, we focused on activity in the afferent fibers at the first synapse in the nucleus of the solitary tract.[10]

Slicing the brain horizontally through the nucleus of the solitary tract in the brainstem leaves the afferent pathway intact, allowing for electrical stimulation of axons on the pathway and measurement of the evoked postsynaptic responses. Placing a small amount of dye on the carotid body allows one to distinguish the cells that receive chemoreceptive input as the dye travels the axons to the presynaptic terminals in the nucleus of the solitary tract.

Using differential interference contrast microscopy, we stimulated the solitary tract under voltage clamp conditions: holding the voltage constant prevents the membrane potential from moving into regions that would activate other ion channels. This enabled us to recognize the responses at specific synapses.[10]

Contrary to our expectations, rats had a dramatically *reduced* evoked synaptic response after 10 days and 30 days of intermittent hypoxia.[10]

As a result of intermittent hypoxia, each action potential arriving from a chemoreceptive afferent fiber releases about half (or even fewer) of the vesicles of neurotransmitter that are normally released. Further experiments verified that the change occurs presynaptically, not in the postsynaptic cell.[10]

### Inhibited response following intermittent hypoxia is reversible

The reduced current that occurs from intermittent hypoxia is reversible. Rats that have been through 10 days of intermittent hypoxia followed by 10 days of living in a normal oxygen environment have a near-control postsynaptic current.

### Intermittent hypoxia induces increased spontaneous activity presynaptically

To mimic continuous activity arising from carotid chemoreceptors, we delivered a series of 20 stimuli to chemosensory afferent fibers.[10] In normal rats, each stimulus produced a synaptic response. Characteristically, the first one is a large response, and the other responses are diminished.

In rats exposed to intermittent hypoxia, stimulation

produced, in addition to the reduced stimulus-evoked response described above, more spontaneous activity between stimuli, indicating that the synapse has become "hyperactive" and is releasing more transmitter. But the afferent activity arriving from the chemoreceptors is less effective in releasing transmitter.

### Intermittent hypoxia alters calcium modulation of transmitter release

Why is the response to stimulation diminished? The spontaneous release of transmitter may deplete the pool available for evoked release. Alternatively, two pools of transmitter may exist that are affected differently by chronic intermittent hypoxia.

Extracellular calcium is directly related to the amount of transmitter released: raising extracellular calcium concentrations increases transmitter release. Presumably, more calcium enters through calcium channels when a fiber is activated, causing more transmitter release. Conversely, reducing calcium concentrations leads to smaller postsynaptic currents.

Chronic intermittent hypoxia appears to affect the handling of calcium presynaptically. In rats exposed to chronic intermittent hypoxia, the entire response is reduced and cannot be enhanced by increasing calcium concentration. However, the response can be further reduced by reducing calcium concentration.

### CaM kinase II is involved in calcium changes of chronic intermittent hypoxia

The amplitude of the synaptic evoked potential returns toward normal in the presence of a blocker of calmodulin (CaM) kinase II, implicating this enzyme in the depression of synaptic response in chronic intermittent hypoxia. We recently found that the amount of the phosphorylated form of calmodulin-dependent protein kinase II (pCaM kinase II) increases in rats exposed to chronic intermittent hypoxia as compared with normoxic animals (data submitted for publication).

### What information is getting through the chemoreflex pathway?

Despite reduced amplitude of the postsynaptic response, overall transmission through the synapse is increased between the chemosensory afferents and the neurons in the nucleus of the solitary tract. The excess spontaneous activity observed can elicit action potentials, so that more activity continues to the next cell in the synaptic pathway, even though the amplitude of individual impulses is reduced.

## FUTURE RESEARCH DIRECTIONS

We believe that the reduced size of the synaptic current may be an adaptive response to tone down the increased activity. Perhaps the calcium defect is more widespread than observed and may also be operating in the carotid chemoreceptors. Whether or not the calcium-induced response can be manipulated to prevent it from occurring is an area of future research.

Other intriguing questions remain:

- How is the increase in chemosensory information conveyed from the nucleus of the solitary tract to the blood vessels? Are there other sites in this pathway where chronic intermittent hypoxia modifies the activity?
- Why is the increase in arterial pressure sustained?
- Why do the baroreceptors not correct for the increase in arterial pressure?
- What causes the hypertension to reverse after normal oxygen conditions are resumed?

## REFERENCES


1. **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.
2. **Somers VK, Dyken ME, Clary MP, Abboud FM.** Sympathetic neural mechanisms in obstructive sleep apnea. J Clin Invest 1995; 96:1897–1904.
3. **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.
4. **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.
5. **Fletcher EC, Lesske J, Culman J, Miller CC, Unger T.** Sympathetic denervation blocks blood pressure elevation in episodic hypoxia. Hypertension 1992; 20:612–619.
6. **Fletcher EC, Lesske J, Behm R, Miller CC 3rd, Stauss H, Unger T.** Carotid chemoreceptors, systemic blood pressure, and chronic episodic hypoxia mimicking sleep apnea. J Appl Physiol 1992; 72:1978–1984.
7. **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.
8. **Kumar GK, Rai V, Sharma SD, et al.** Chronic intermittent hypoxia induces hypoxia-evoked catecholamine efflux in adult rat adrenal medulla via oxidative stress. J Physiol 2006; 575:229–239.
9. **Hinojosa-Laborde C, Mifflin SW.** Sex differences in blood pressure response to intermittent hypoxia in rats. Hypertension 2005; 46:1016–1021.
10. **Kline DD, Kunze DL.** Alterations in neurotransmitter release properties occur in the nucleus of the solitary tract following intermittent hypoxia. Abstract 283.15. Presented at: 34th Annual Meeting of the Society for Neuroscience; October 2004; San Diego, CA.


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