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Intermittent hypoxia: cell to system

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Prabhakar, Nanduri R., R. Douglas Fields, Tracy Baker, and Eugene C. Fletcher. Intermittent hypoxia: cell to system. Am J Physiol Lung Cell Mol Physiol 281: L524–L528, 2001.—This symposium was organized to present research dealing with the effects of intermittent hypoxia on cardiorespiratory systems and cellular mechanisms. The pattern of neural impulse activity has been shown to be critical in the induction of genes in neuronal cells and involves distinct signaling pathways. Mechanisms associated with different patterns of intermittent hypoxia might share similar mechanisms. Chronic intermittent hypoxia selectively augments carotid body sensitivity to hypoxia and causes long-lasting activation of sensory discharge. Intermittent hypoxia also activates hypoxia-inducible factor-1. Reactive oxygen species are critical in altering carotid body function and hypoxia-inducible factor-1 activation caused by intermittent hypoxia. Blockade of serotonin function in the spinal cord prevents long-term facilitation in respiratory motor output elicited by episodic hypoxia and requires de novo protein synthesis. Chronic intermittent hypoxia leads to sustained elevation in arterial blood pressure and is associated with up-regulation of catecholaminergic and renin-angiotensin systems and down-regulation of nitric oxide synthases.

protein kinases; gene expression; carotid body chemoreceptors; respiratory plasticity; blood pressure control

RECURRENT EPISODES OF HYPOXIA are encountered more often in life and are associated with many pathophysiological situations including sleep apneas and apneas in premature infants and with chronic obstructive pulmonary disease, asthma, or pulmonary fibrosis (see Ref. 21 for references). Chronic intermittent hypoxia leads to serious pathophysiological situations. Examples of the consequences of chronic intermittent hypoxia include pulmonary as well as systemic hypertension, myocardial and brain infarctions, and cognitive dysfunction. This symposium was organized to present some aspects of the current research dealing with the effects of intermittent hypoxia on cardiorespiratory systems and the cellular mechanisms associated with these changes. The focuses of the symposium were 1) how the pattern of stimulus influences the gene expression in neuronal cells, 2) what are the consequences of intermittent hypoxia on the oxygen-sensing ability of the carotid body chemoreceptors and transcription factor activation, 3) the mechanisms associated with plasticity of respiratory output induced by intermittent hypoxia, and 4) the mechanisms associated with elevated blood pressure caused by chronic intermittent hypoxia.

PERIODIC CHANGES IN INTRACELLULAR CALCIUM IN REGULATING CELL SIGNALING AND GENE EXPRESSION

Many adaptive responses to hypoxia involve the regulation of specific genes, and these responses differ

1 Presented by R. Douglas Fields.

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depending on whether hypoxia is acute, chronic, or intermittent. Transcription of these genes is regulated by intracellular signaling pathways involving Ca\(^{2+}\) influx through L-type voltage-sensitive Ca\(^{2+}\) channels, protein kinases, including Ca\(^{2+}\)/calmodulin-dependent kinase II (CaMKII) and mitogen-activated protein kinase (MAPK), which regulate gene transcription via phosphorylation of transcription factors such as cAMP-responsive element binding protein (CREB). Immediate-early genes such as c-fos control transcription by binding to regulatory elements in the promoter region of late-response genes. There are many similarities between the regulation of gene transcription during intermittent hypoxia and how the brain develops and modifies its structure and function through experience. The structure and function of the nervous system are controlled, in part, by functional activity arising from sensory input. Information in the environment is coded in the temporal pattern of action potential firing. Fields et al. (10) investigated the hypothesis that the transcription of genes in neurons can be regulated by the pattern of neural impulse activity and examined the intracellular signaling mechanisms that transduce and integrates information from patterned membrane depolarization to control the transcription of genes. Studies were performed on neurons cultured from the mouse dorsal root ganglion in a culture chamber equipped with electrodes for electrical stimulation. mRNA levels of cell adhesion molecules, for example L1 and NCAD, are regulated by specific frequencies of action potentials. L1 is downregulated by 0.1-Hz stimulation, but 1-Hz stimulation is not effective, whereas other patterns of stimulation are effective in regulating expression of other cell adhesion molecules (17). Functional effects, including changes in cell-cell adhesion, axon fasciculation, and myelination, are induced by patterns of action potential firing that regulate L1 expression.

The immediate-early gene c-fos was chosen as a model for studying the mechanism for transcriptional regulation by appropriate patterns of stimulation (23). This work involved tracing the cell signaling pathways from Ca\(^{2+}\) influx through voltage-sensitive Ca\(^{2+}\) channels through the Ca\(^{2+}\)-dependent kinases and transcription factors that regulate transcription of this gene. The relationship between Ca\(^{2+}\) dynamics and gene expression indicated that the temporal dynamics of Ca\(^{2+}\) transients are more important than the concentration levels (9). Even small periodic changes in Ca\(^{2+}\) (20 nM) can induce transcription of this gene if repeated at an appropriate frequency (10 s), whereas large changes in Ca\(^{2+}\) repeated at inappropriate intervals (120 s) do not stimulate transcription significantly. The hypothesis that the autonomous activation of CaMKII could decode the frequency of Ca\(^{2+}\) pulses was tested with an in vitro kinase assay. The frequency-decoding properties of CaMKII were limited to frequencies between 0.1 and 1 Hz (10). This is due, in part, to the high levels of autonomous activation of the enzyme in the basal state and the prolonged duration of the Ca\(^{2+}\) transients induced by action potentials rather than by a single molecular “Ca\(^{2+}\) pulse frequency detector.” These observations suggest that the network of intracellular signaling pathways acting as a system can decode the temporal pattern of stimuli to activate the appropriate transcriptional responses. This derives, in part, from differences in the kinetics of individual signaling reactions in the intracellular signaling network. For example, the kinetics of CREB phosphorylation are rapid, but the dephosphorylation kinetics are slow (requiring tens of minutes). In contrast, MAPK phosphorylation is rapid, but this kinase dephosphorylates much more rapidly (9). This implies that periodic increases in intracellular Ca\(^{2+}\) separated by long periods of inactivity could propagate through signaling pathways involving CREB but that more frequent periodic increases in Ca\(^{2+}\) would be required to propagate signals through the intracellular signaling pathways involving MAPK. The measurements of c-fos transcription are consistent with such a possibility, and this mechanism may help explain how periodic stimulation of appropriate frequencies can activate transcription of the appropriate genes (7).

Although these investigations described above focused on periodic action potential stimuli during development of the nervous system, similar mechanisms might be operating in eliciting genomic responses to other periodic stimuli, including intermittent hypoxia.

MECHANISMS ASSOCIATED WITH ALTERATIONS IN CAROTID BODY FUNCTION AND TRANSCRIPTION FACTOR ACTIVATION BY INTERMITTENT HYPOXIA

Much of the information on the long-term effects of intermittent hypoxia on the ventilatory control system has come from studies on patients with recurrent apneas (sleep or central apneas). Humans with sleep apneas exhibit an enhanced ventilatory response to hypoxia, whereas respiratory responses to hypercapnia are less affected, unaltered, or even blunted (8). It has been suggested that carotid body chemoreceptors constitute the “frontline” defense system for detecting changes in arterial blood gases during apneas. However, there have been no studies that examined the long-term effects of intermittent hypoxia on carotid body activity. Peng and colleagues (18, 19) examined the long-term effects of intermittent hypoxia (a pattern similar to recurrent apneas) on carotid body activity in rats. Awake rats were exposed to 15 s of 5% O\(_2\) followed by 5 min of 21% O\(_2\) (9 episodes/h, 8 h/day, for 10 days). Carotid body sensory activity was subsequently examined in anesthetized, paralyzed, and mechanically ventilated rats. It was found that the baseline sensory discharge of the carotid bodies and the magnitude of the sensory response to hypoxia were greater in rats exposed to 10 days of intermittent hypoxia. Similar potentiation of the hypoxic sensory response was also seen in an in vitro carotid body preparation, suggesting that the enhanced carotid body sensitivity to hypoxia is not secondary to alterations in blood pressure and/or

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\(^2\) Presented by Nanduri R. Prabhakar.
circulating vasoactive hormones. However, the carotid body sensory response to hyperoxic hypercapnia (5% CO2, 95% O2) was found to be unaffected by intermittent hypoxia, suggesting that the carotid body sensory response to hypoxia, but not to CO2, is selectively enhanced in response to long-term intermittent hypoxia. It is likely that the enhanced chemoreceptor sensitivity led to hyperventilation, thus driving the respiratory controller below the apneic threshold for O2, resulting in more apneas. Thus the enhanced carotid body sensitivity to hypoxia might act as “positive feedback,” exacerbating the occurrence of apneas.

After repeated episodes of hypoxia, breathing remains elevated for nearly 1 h, and this phenomenon is often termed long-term facilitation (LTF) and requires afferent input from the carotid bodies (20). Whether episodic hypoxia causes LTF in the carotid body sensory discharge, however, has not been examined. To test this possibility, anesthetized rats were subjected to 10 episodes of hypoxia (12% O2) each episode lasting 15 s interspersed with 5 min of normoxia. As expected, carotid body activity increased in response to each episode of hypoxia. However, after the last episode of hypoxia was terminated, the baseline discharge promptly returned back to the control level. By contrast, episodic hypoxia resulted in a prolonged elevation in baseline carotid body sensory activity that lasted nearly 1 h in animals conditioned with 10 days of intermittent hypoxia. This increase in baseline activity after episodic hypoxia occurred despite the maintenance of arterial blood pressure and blood gases. These observations suggest that conditioning with intermittent hypoxia induces LTF in carotid body sensory activity. LTF in carotid body activity might lead to stimulation of the sympathetic nervous system, causing a sustained elevation in blood pressure.

It is likely that the long-term effects of intermittent hypoxia on the carotid body involve the increased generation of reactive oxygen species (ROS) because intermittent hypoxia resembles ischemia-reperfusion. During reperfusion, the cellular generation of ROS increases. To test the possible involvement of ROS, rats were injected with manganese (III) tetrakis(1-methyl-4-pyridyl)porphyrin pentachloride (MnTMPyP; 1 mg·kg−1·day−1), a potent scavenger of superoxide ions, and were then subjected to intermittent hypoxia for 10 days. In these animals, 10 days of intermittent hypoxia did not result in enhanced hypoxic sensitivity of the carotid body. Also, the magnitude of LTF in the sensory discharge was markedly attenuated. Taken together, these observations support the idea that ROS might be playing an essential role in intermittent hypoxia-induced alterations in the carotid body function.

It is being increasingly recognized that activation of specific genes is an important mechanism by which sustained hypoxia triggers long-term adaptive responses. A number of studies have shown that hypoxia-inducible factor (HIF)-1 mediates transcriptional regulation of genes that encode proteins associated with maintaining O2 homeostasis (reviewed in Ref. 22). HIF-1 is a heterodimer consisting of HIF-1α and HIF-1β, also known as the aryl hydrocarbon nuclear translocator subunits. Of the two subunits, the expression of HIF-1α is tightly regulated by O2, whereas HIF-1β is constitutively expressed. The effect of intermittent hypoxia on HIF-1 and HIF-2α protein expression was examined in PC12 cells. Intermittent hypoxia increased both HIF-1 and HIF-2α expression. Reporter gene assay [with the reporter gene driven by the hypoxia response element (HRE)] revealed that intermittent hypoxia increases HIF-1-mediated transcriptional activation. Scavengers of superoxide ions attenuated HRE luciferase activation caused by intermittent hypoxia (25), suggesting that ROS also play an essential role in HIF-1 activation by intermittent hypoxia. Neither MAPK nor phosphatidylinositol 3-kinase inhibitors prevented HRE reporter gene activation by intermittent hypoxia, whereas 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid tetra(acetoxymethyl) ester (a Ca2+ chelator) or Ca2+/CaM kinase inhibitors prevented HRE luciferase activation by intermittent hypoxia. Thus these studies, although preliminary, suggest that Ca2+ signaling pathways are associated with HIF-1 transcription factor activation by intermittent hypoxia in PC12 cells.

**MECHANISMS ASSOCIATED WITH LTF OF RESPIRATORY MOTOR OUTPUT INTERMITTENT HYPOXIA**

Intermittent hypoxia elicits a progressive increase in respiratory motor output termed LTF (1, 2). LTF is a serotonin-dependent form of plasticity that primarily occurs in the central nervous system (1, 16). One key question has been, where in the central nervous system is activation of serotonin receptors required to elicit LTF? Several possibilities exist, including activation of serotonergic receptors at the level of 1) brain stem neurons, generating a respiratory rhythm and pattern, or 2) respiratory motoneurons. To begin to differentiate between these possibilities, Baker and Mitchell (4) applied a broad-spectrum serotonin receptor antagonist locally to the spinal cord of anesthetized rats before intermittent hypoxia. Blocking serotonin function in the spinal cord abolished LTF. These data, combined with results from an earlier study (1) showing that serotonin receptors are required during but not after intermittent hypoxia, suggest that activation of serotonergic receptors in the spinal cord during hypoxia initiates (but does not maintain) a process that facilitates respiratory motor output. At least part of this process involves spinal protein synthesis because localized injections of protein synthesis inhibitors to the spinal cord abolish LTF (3). The newly synthesized proteins that are necessary for LTF are unknown. However, one protein with the requisite characteristics to mediate LTF is the neurotrophin brain-derived neurotrophic factor (BDNF). One hour after intermittent hypoxia, BDNF is increased in the ventral cervical spinal cord, and like LTF, this BDNF increase requires

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3 Presented by Tracy Baker.
spinal serotonin receptor activation and spinal protein synthesis.

Understanding the mechanisms giving rise to LTF may allow important insights concerning plasticity in respiratory motor control and how breathing can change over time to meet changing environmental and physiological conditions. Furthermore, through a better understanding of the capacity for change, particularly in the ventral spinal cord where few examples of plasticity are known, new therapeutic approaches may be developed to compensate for diseases of the respiratory system and spinal cord.

MECHANISM OF SYSTEMIC BLOOD PRESSURE: ELEVATION IN CHRONIC INTERMITTENT HYPOXIA

The systemic arterial blood pressure response in humans to obstructive sleep apnea is acute elevation, returning to near baseline after the apnea. Many investigators believe that the chronic diurnal elevation of blood pressure may follow repetitive nightly apneas, resulting in a sustained systemic hypertension in ~50–70% of sleep apnea patients. Mechanisms of chronic blood pressure elevation are difficult to investigate in humans because of the long duration necessary for subtle stimuli such as recurrent apneas or hypoxia to cause a sustained daytime hypertension. The rat is an unusually good model in which to investigate proposed mechanisms of hypertension because of its short life span and the volume of data on other types of hypertension in rodents. Episodic hypoxia (~1 min) is one of the most prominent features of recurrent apnea associated with profound cardiovascular events even in the absence of apnea. Fletcher et al. (13) examined the response to chronic episodic hypoxia lasting ~15 s, recurring every 30 s for 8 h/day over a consecutive 35-day period in several strains of rats. They discovered that elevation of systemic blood pressure is sustained for several weeks (in the absence of hypoxic stimulation) after the period of exposure. The cycles of hypoxia may be applied repetitively over weeks, for variable periods (e.g., 8–24 h) during the day, or with varying concentrations of added CO₂, simulating the recurrent episodic hypoxia or asphyxia of sleep apnea.

Thus far, the findings by Bao and Fletcher (5) and Fletcher et al. (11) indicate that acute and chronic episodic hypoxia recurrently stimulate the peripheral chemoreceptors as evidenced by elimination of the chronic elevated blood pressure response in carotid body-denervated, episodic hypoxia-exposed rats. The diurnal increase in blood pressure is also blocked by chemical peripheral sympathectomy (12). Further support for this possibility is demonstrated by increased plasma norepinephrine as well as increased right ventricular myocardial catecholamines in episodic hypoxia-exposed rats (12). Sympathetic blockade by prazosin blunts the acute blood pressure response to episodic hypoxia, and direct recording of splanchnic nerve activity in the awake, unrestrained rat confirms that hypercarbia combined with episodic hypoxia causes a profound increase in sympathetic activity (6). It appears that the adrenal gland as well as renal sympathetic nerves participates in the chronic diurnal blood pressure elevation (6). These two end organs of sympathetic activity may act synergistically in the setting of episodic hypoxia by adrenal release of epinephrine, which binds to peripheral sympathetic nervous system synapses, potentiating neurotransmission. Second, there may be facilitation of the release of renin through α-receptors in the kidney. Supporting this is the demonstration of increased renin-angiotensin system activation in episodic hypoxia-exposed rats as well as inhibition of the blood pressure response to episodic hypoxia by angiotensin type I receptor blockers (14). A high-salt diet (volume expansion with suppression of renal sympathetic nerves and renin) blocks the blood pressure elevation and suppresses kidney tissue renin mRNA in chronic episodic hypoxia-exposed rats (Fletcher EC, Orlonova N, and Bader M, unpublished observations). This further emphasizes the importance of the sympathetic nervous system and the renin-angiotensin system in the mechanism of hypoxia-induced hypertension. Tahawi et al. (24) have tested microvascular tone in a cremaster skeletal muscle preparation after 35 days of episodic hypoxia. They demonstrated increased arteriolar vascular tone as evidenced by decreased acetylcholine dose-response curves and increased vasoconstriction in the presence of N-nitro-l-arginine methyl ester. The exact mechanism for this remains unknown. There is the possibility that episodic hypoxia modulates vascular tone through direct action on the vascular endothelium.

REFERENCES


4 Presented by Eugene C. Fletcher.


