Historical perspectives of cellular oxygen sensing and responses to hypoxia

SUKHAMAY LAHIRI
Department of Physiology, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania 19104-6085

Lahiri, Sukhamay. Historical perspectives of cellular oxygen sensing and responses to hypoxia. J Appl Physiol 88: 1467–1473, 2000.—The responses to acute and chronic hypoxia begin with oxygen sensing, and this historical perspective is written in line with this concept. The earliest pertinent work started with studies on fermentation in yeast in the 17th century, before the discovery of oxygen. It required 200 yr to localize the oxygen sensing within the cells and another 100 yr to discover the cellular oxidation reactions. Today, the consensus is that the mitochondrial respiratory chain is in part the site of oxygen sensing. In addition, membrane-bound NAD(P)H oxidase possibly takes part in oxygen sensing. Oxygen-sensing mechanisms occur in a tissue-specific fashion. For example, the carotid body responds to hypoxia promptly by eliciting a ventilatory response, whereas erythropoietin production in response to hypoxia requires more time, involving new expression of genes. The mechanism has therefore moved from the cells to genes.

Invited historical perspective on “Hypoxia Influence on Gene Expression.”

http://www.jap.org 8750-7587/00 $5.00 Copyright © 2000 the American Physiological Society
This historical perspective will first describe how oxygen’s discovery began with fermentation in yeast. Next, intracellular respiration and oxidation are described. This leads to the responses to hypoxia, including the tissue-specific responses. Next, the carotid body and several hypotheses for the oxygen-sensing mechanisms are discussed. Liver and kidney cells are then dealt with, leading up to erythropoietin production. Vascular responses are next described. Finally, the hypothesis that the sensor is a heme protein is considered.

FERMENTATION AND RESPIRATION: USE OF YEAST

Yeast is an interesting unicellular organism that has been known to man from time immemorial. Using his microscope, Leeuwenhoek (1632–1723) first observed that brewer’s yeast is composed of small globules that formed gas bubbles in the absence of oxygen. However, it was not until 1838 that Cagnard-Latour found the relationship of fermentation (anaerobic metabolic gas bubble formation) to the growth and multiplication of yeast in sugar as seen under the microscope. The next significant advancement in the study of fermentation was made by Pasteur (1822–1895), and this had great repercussions on different branches of biology, medicine, and industry. He reported that living microorganisms could proliferate in both the presence and absence of oxygen. In the presence of oxygen, yeast cells display little fermentation, but in the absence of oxygen they showed active fermentation. This relationship between fermentation and respiration applies not only to microorganisms but to all cells.

Bernard (1813–1878) also studied fermentation, but his view differed from that of Pasteur’s in that he considered fermentation not to be a vital process but due to a chemical cause and requiring further investigation. His view was substantiated by Edward Buchner in 1897 some 20 yr after the death of Bernard and 2 yr after that of Pasteur. Buchner showed that an extract of yeast, free of cells, could ferment sugar and produce alcohol and CO₂. This discovery stimulated subsequent work on intracellular oxidation-reduction reactions.

Warburg (1883–1970) also worked with yeast and confirmed the observations of Pasteur on the reduction of fermentation by oxygen, which he called the Pasteur effect. He found that inhibition of respiration by CO increases fermentation by yeast, and the inhibition could be reversed by a so-called action spectrum of light of different wavelengths, thus producing the first photochemical absorption spectra and identifying the different components of cytochromes involved (21). The action spectra of yeast inhibition by CO offered a new and very valuable method for the study of the mechanism of cell respiration (12).

Yeast continued to provide even greater avenues for research, including the mechanisms for oxygen sensing in unicellular organisms. Some investigators (13) used CO to explore oxygen sensing in yeast. Half of their experiments pointed to cytochrome oxidase as the oxygen sensor, but how the oxygen sensing was linked to genes was not clear.

DISCOVERY OF OXYGEN LEADING UP TO INTRACELLULAR RESPIRATION

In tracing the history of cellular oxygen sensing and responses, the difficulties and achievements in the development of several lines of knowledge become apparent. The phlogiston theory of Stahl (1660–1734) dominated the thinking of scientists about respiration in the 18th century to such a degree that Joseph Priestley (1773–1804) thought that the purpose of breathing consisted not of receiving something from air but in giving off something, i.e., phlogiston to dephlogisticated air. He made the brilliant observation that mice could not survive in air “injured” by a candle flame but that this air could be restored by a sprig of leaves, thus allowing mice to survive. He had his own reasons for introducing the leaves and almost missed the discovery of oxygen and, in retrospect, photosynthesis all because of his strong faith in phlogiston. Later, when Richard Kirwan (1733–1812) denounced the doctrine of phlogiston, Priestley recognized his courage and scientific integrity.

After 20 yr of fighting against Stahl’s phlogiston theory, Antoine Laurent Lavoisier (1743–1794) came out clearly in favor of oxygen in air and stated that respiration is nothing but a slow combustion of carbon and hydrogen with oxygen. Nevertheless, he still believed that respiration occurred in the blood in the lungs.

It was Lazzaro Spallanzani (1729–1799) who made the next advance, localizing oxygen uptake and CO₂ output in the peripheral tissues rather than in blood in the lungs. However, it was another 100 yr before Eduard Pflüger (1829–1910) provided a solid foundation for the concept that respiration was an intracellular process. At this time, Claude Bernard (1813–1878) turned his mind to respiration and fermentation. He ultimately agreed with Pflüger’s view that respiration is localized within the cells. However, his concept was that energy and heat were derived from continual destruction of the tissues themselves. Bernard’s views on the mechanism of tissue respiration were very much opposed to those of Lavoisier. Ridiculing Lavoisier, he wrote that his theory was “illuminating in its principle but faulty in its expression, consisted in equating respiration with true combustion and in locating the site of this combustion in the lungs” and that his achievement was “great enough for the glory of the most ambitious man” (12). However, Paul Bert (1873–1886), a pupil of Bernard, clarified the issue and stated that it was the foodstuffs and not living tissues that underwent combustion in the tissues. However, he did not have any clue as to the intracellular oxidation reaction.

Mechanisms of Cellular Oxidation

MacMunn (1852–1911) made the first observation on intracellular oxidations. MacMunn’s (1884) observations on myohematin and histohematin anticipated studies of cytochromes, but his work was ignored by many leaders in the field. Later Keilin (summarized in
Ref. 12) tried to give him credit, although apparently unsuccessfully. MacMunn’s observation was based on the spectroscopic examination of different tissues. In all of these, he found the characteristic four-banded absorption spectra of a pigment in the reduced state that disappeared in the oxidized state, and he associated this observation with the respiratory processes. Although this observation was essentially correct, Keilin pointed out some difficulties that hindered acceptance of MacMunn’s work. The chief problem was MacMunn’s inability to explain the four absorption bands present in the reduced form and his inability to demonstrate that these pigments underwent reversible oxidation and reduction in living cells (see explanation below).

Warburg’s (1883–1970) work on cellular respiration and biological oxidation began in 1908 and primarily continued through 1925. He described an iron-containing “Atmungsferment” (respiratory enzyme) and won the Nobel Prize in 1931 for this work (21). According to his views, cellular respiration was a surface reaction that consisted of unspecific surface forces and specific chemical forces. The combustible substances condensed onto the cell surface, and the absorbing surface contained a substance that transported oxygen by means of chemical forces. This substance was the respiratory ferment (“Atmungsferment”), containing iron, which in its bivalent state reacted with molecular oxygen. The oxidized iron reacted with substrate and reverted back to the bivalent state. This theory of biological oxidation and respiration received several criticisms and started a lively controversy between David Keilin and Otto Warburg (see Ref. 12). Incidentally, the word “atmung” or “atmen” sounds like “atman,” meaning to breathe, described in the Upanishads.

Keilin identified MacMunn’s histohematin in a far greater variety of cells and aptly named this “cytochrome,” signifying cellular pigment. This name was rapidly accepted in the literature (1925) and has persisted ever since. It was found not only in the cells of animals but also in yeast, bacteria, and plant cells. It is now well known for its general structure and function in cellular respiration. The cytochrome is a system consisting of three chromogens, unlike MacMunn’s single pigment, which explains the nature of the four-banded absorption spectra that baffled MacMunn. It is fundamentally different from myoglobin and hemoglobin, removing the confusion that previously existed.

Warburg’s views on cytochrome in relation to respiration were different and often changed. At one point (1928), he applied the term “degenerate ferment” to cytochrome and was surprised to see that cytochrome could be so easily observed in such a considerable quantity in living cells, suggesting that it could not be a ferment. Later, in his published Nobel lecture (1932), he expressed doubt about assigning cytochromes to cellular respiration, although most people involved in intracellular respiratory metabolism and catalysis accepted it. Cytochrome, however, appeared in papers by Warburg in 1933–1934. By 1946, Warburg used the term “cytochrome oxidase” in his book in accordance with Keilin’s wishes (see Ref. 21). Peter Mitchell (1920–1992) provided the final link between oxygen reduction and ATP production with his theory of biological energy transfer, driven by a proton gradient in the mitochondria, for which he won the Nobel Prize in 1978.

**RESPONSES TO HYPOXIA**

Most of the oxygen consumption of a cell depends on its mitochondrial metabolism. Oxygen consumption decreases with a decrease of PO2 below a “critical” value. The critical PO2 in the resting cells varies between 10 and 15 Torr (18), although it is below 1 Torr in the isolated mitochondria (4). It is known, however, that at the summit of Mount Everest where the inspired PO2 was found to be 43 Torr, with resting alveolar PCO2 of 10 Torr and alveolar PO2 of ~30 Torr, human subjects can hardly survive. In the acute phase, they become unconscious. Chronically, this degree of hypoxia is intolerable.

Not all cells respond to hypoxia to the same degree or with the same rapidity, although they have a common mechanism for oxygen reduction. Some respond without delay (acute response), whereas others respond with considerable delay, requiring altered gene expression (chronic response). All cells that have mitochondria (yeast and animal cells) and NAD(P)H oxidase are expected to respond rapidly, but the cellular expression might occur at a different tissue PO2 or be delayed. For example, the carotid body response begins at a much higher PO2 than that for pheochromocytoma (PC-12) cells and more rapidly than the response of erythropoietin production in the kidney and liver. The tissue-specific responses indicate that there are intermediate carriers for the responses. The historical developments of these tissue-specific cellular effects are of interest.

**Tissue-Specific Responses**

Mammalian cells (3, 5, 6, 9, 11) and yeast cells (13) show oxygen sensing. Glomus-like cell types are present in the carotid body, aortic bodies, neuroepithelial bodies, abdominal ganglia, and pheochromocytoma (PC-12) cells (25). Of these, only the cells from the carotid body, neuroepithelial body, and PC-12 cells have been studied electrophysiologically and show similar behavior (3, 5, 6, 9, 11). However, because they are devoid of sodium channels, these cells seldom fire (17). Even the carotid body cells do not fire but their afferents do. A major difference between these cells and those that are electrically excitable is that these cells contain, for example, dopamine, norepinephrine, serotonin, and ACh, suitable for secretory cells. Their nerve endings may have special mechanisms for oxygen sensing, generating action potentials. The study of the nerve endings in the carotid body has proved to be difficult (6), but indirect evidence has been accumulating (16).

**Peripheral Chemoreceptors: Ventilatory Responses**

A great many of these responses are known (9), and they provide prototypes for other tissues and are therefore dealt with here in greater detail. The carotid body, the aortic body, and neuroepithelial bodies are included...
in the peripheral chemoreceptors, all of which contain glomus type I cells, which in turn are innervated by nerve afferents. Several of these units are enveloped by another type of cell, the type II cell. The nerve afferents carry messages from the complex to the central nervous system. In the case of the carotid body, they run in the carotid sinus nerve and produce the well-known respiratory and cardiovascular reflex responses. The afferents from the aortic bodies run in the vagus nerves and are responsible for similar but weaker responses. The afferents from the neuroepithelial bodies also run in the vagus nerves but their function is not known.

Carotid body. Because of uncertain documentation, the initial history of its discovery is shrouded in mystery (7). It began in the early 18th century when the phlogiston theory of lung gas exchange dominated the scene. The first description of the carotid body appeared in a dissertation in 1743 by Hartwig Wilhelm Ludwig Taube. Then, after several rediscoveries, Hubert Luschka (1820–1875) renamed it glandula carotica.

About a century later, Fernando de Castro of the Cajal Institute of Madrid produced a histological description of the carotid body (1927, 1928), which has survived the test of time. From its vascular structure and innervation, he intuitively proposed that it was a sensory organ, “tasting blood.” The Spanish Civil War interrupted his work until 1940. Afterward, he extended his work to include the carotid sinus baroreceptor, returning in 1960 to the electron microscopy of the carotid body, only a short time before his death.

Although de Castro’s study established the anatomic structure of the carotid body, there was another group, primarily consisting of father and son, the Heymans, who were exploring the physiological and pharmacological significance of the structures using cross-circulation experiments in dogs. One day, after doing a planned experiment, Cornelle Heymans performed a “foolish” experiment on a dog in which the neural connection from one sinus nerve had been cut while the other sinus nerve remained intact. They injected potassium cyanide into the common carotid artery, which produced tremendous hyperventilation, which did not occur after the sinus nerve was cut. Subsequently, they repeated this observation in several “planned” experiments. That was the genesis of the superb experiments of Cornelle Heymans, for which he was awarded the Nobel Prize in 1938 (see Ref. 7).

Ever since the discovery of the importance of the carotid body and the carotid sinus nerve in the control of ventilation, it has been the respiratory physiologists and associated neurophysiologists who have been primarily involved in studies of chemoreceptor function, and this domination continues today. There are several hypotheses of chemoreception, some of which are enumerated below.

Mitochondrial respiratory chain hypothesis. The respiratory electron transport chain of mitochondria is the seat of the major oxygen consumption and therefore is a likely source of oxygen signaling. Thus the stimulation of the chemoreceptors by inhibitors of oxidative metabolism is taken as evidence for oxygen signaling (2). Single-fiber studies of chemoreceptor afferents showed that the same fibers responded to both hypoxic and CO₂/H⁺ stimuli, that the effect of one was augmented by the other, and that threshold for one was lowered by the other (14). These investigators recognized that their findings were similar to the effect of CO₂/H⁺ on the hemoglobin-oxygen dissociation curve, the Bohr effect, and postulated that a hemoglobin-like receptor was involved in the chemoreception process. They also found that, when CO was added to an in vitro hemoglobin-free carotid body preparation that was perfused and superfused, it mimicked the hypoxic response in the dark, and the effect with normoxic fluid could be eliminated by focussed bright white light. They also went on to find that the action spectra, as per Warburg, mimicked that of cytochrome oxidase. Because CO is expected to bind to heme compounds, this finding indicated that the heme of cytochrome oxidase was the key for this response (23). Of course, CO inhibited oxygen uptake in the dark, which was relieved by light (15), as had been found with yeast cells (12), presumably because light dissociated the combination of CO with heme ligand.

More recently, Lahiri et al. (16) confirmed these findings and added a new dimension. When measuring the redox state of cytochromes and the chemoreceptor discharge of the rat carotid body simultaneously under the influence of CO, they found that chemoreceptor discharge peaked when the cytochromes were only slightly reduced. As the cytochromes became more reduced, the discharge rate declined. At this stage when hypoxia had reached an anoxic state, chemoreceptor activation by CO decreased, and the effect of light was reversed. This reversal of the light effect was thought to be caused by the decrease in oxygen metabolism in the dark so that there was insufficient chemical energy to activate the neural signal. The respiratory chain hypothesis was also supported by Mills and J obsis and Bisocce and Duchen (see Ref. 9).

Membrane channel hypothesis. This hypothesis originated most recently with the advent of patch-clamp studies of membrane channels (17). The consensus model is that hypoxia and hypercapnia reduce the outward potassium currents in the glomus cell membrane, which depolarizes these chemoreceptor cells, opening up voltage-gated calcium channels and allowing calcium entry into the cell, thus causing the neurotransmitter release. However, classic potassium current inhibitors influence neither chemosensory discharge nor the calcium concentration of the cells (19). The mechanism of the hypoxic effect on potassium currents is not known, but it is supposed to involve heme-containing membrane protein. Other voltage-sensitive human ether-a-go-go-related gene (HERG)-like potassium currents or voltage-insensitive potassium leak currents have been postulated (9, 16), but information is scarce.

Acid hypothesis. In 1937, Winder proposed that hypoxic stimuli increased extracellular acidity, which generated the neural discharge. In 1968, Torrance revived the hypothesis in several forms, eventually
proposing acid formation in the cell (see Ref. 9). However, hypoxia has not been found to make the chemoreceptor cells acid (see Ref. 14).

NAD(P)H oxidase hypothesis. Most of the oxygen consumed in metabolism is reduced to HOH, a four-electron reaction, in the electron transport chain of the mitochondria, with no formation of ROS intermediates. However, there are other enzymes present in tissues that can form superoxide oxygen, a single-electron reduction, or H$_2$O$_2$, a two-electron reduction, both of which are highly reactive species. The production of these ROS is accelerated by high oxygen pressure, and this is considered to be the reason why oxygen is toxic for most organisms at some level (8).

Phagocytes contain a membrane-bound NAD(P)H oxidase, which, when the cells are stimulated, increases their oxygen consumption as much as 20-fold, reducing oxygen to O$_2$,. This last oxidizes chloride to hypochlorite, killing bacterial and fungi. This is a potential oxygen sensor, and it has been postulated that ROS are formed during acute and chronic hypoxia (1). Acker (1) tested this hypothesis of oxygen sensing in the carotid body. He found that NAD(P)H oxidase participates in oxygen sensing in the carotid body and Hep 3B cells of liver. A similar hypothesis has been proposed for neuroepithelial bodies (5).

Neuroepithelial Bodies

Lauweryns was the first to describe the cluster of innervated cells in the pulmonary airways named neuroepithelial bodies, and he continued to study them until 1990. Cutz and Jackson (5) in summarizing their findings from 1993 onward stated that the glomus cells contained serotonin, substance P, calcitonin gene-related peptide, and so forth, but no dopamine, norepinephrine, or ACh, unlike carotid body glomus cells. These cell membranes also showed oxygen-sensitive potassium currents, and NAD(P)H oxidase is present; however, their responses resemble those of secretory cells. It is possible that an endocrine function of these cells is combined with and modulated a chemoreception function.

Integrative Model for Oxygen Sensing in the Carotid Body

The structural model of the chemoreceptor is the presynaptic type I glomus cell synaptically connected with afferents, which have cell bodies in the petrosal ganglion. As a result of a stimulus, a rise in intracellular free calcium is produced, which is essential for the release of neurotransmitters that act on the nerve endings to generate action potentials. Although the cell is full of neurotransmitter-like substances, the actual neurotransmitters have not been identified. According to one model, the cells are depolarized by hypoxia or by hypercapnia and voltage-gated calcium channels open, allowing calcium entry. The extracellular calcium concentration is in the 2–3 mM range, whereas resting intracellular calcium is ~100 nM and can rise to 1,000 nM or more on stimulation. Intracellular calcium can potentially rise even further. In addition, there are intracellular calcium stores that can release free calcium without cell depolarization, and this then acts on the vesicles to induce neurotransmitter release. But how oxygen is sensed in the first place is not known.

ERYTHROPOIETIN PRODUCTION

Production of erythropoietin, which augments red cell mass in response to hypoxia, is due to new expression of genes in the kidney and liver cells (3). These are the only mammalian cells that require changes in gene expression before a response occurs (10). All other cells respond acutely before a chronic response is seen. According to the generally accepted model, after oxygen is sensed by way of the mitochondrial respiratory chain or NAD(P)H oxidase reaction containing iron, hypoxia-inducible factor (HIF-1α) is activated.

**Fig. 1. Model of cellular oxygen sensing and responses.** Acute responses to hypoxia (left) occur through mitochondria and membrane-bound NAD(P)H oxidase. They involve Ca$^{2+}$ rise and shutdown of membrane K$^+$ and opening of Ca$^{2+}$ channels. Chronic effects of hypoxia (right) work through the same mechanisms but involve hypoxia-inducible factor (HIF)-1 formation, which requires time and which then is translocated into nucleus with subsequent activation of a battery of genes.
VAScular oxygen SENSING AND RESPONSES

Pulmonary vasculature constricts due to hypoxia (23), a phenomenon first observed by von Euler and Liljestrand (1946). Since then, the literature on the subject has grown enormously. Although the mechanisms are not clear, the hypoxic constriction appears to depend partly on NAD(P)H oxidase and ROS (22). Space limitation will not allow elaboration of this system.

THE SENSOR IS A HEME-PROTEIN

The effects of CO in various tissues, as indicated previously, provide evidence for heme-protein as the oxygen sensor molecule. The acute effect at high PCO levels is stimulatory through the mitochondrial respiratory chain (24), but the chronic effects become inhibitory due to reactions in the membrane as seen in the suppression of expression of erythropoietin, vascular endothelial growth factor, phosphoendo pyruvate, and carboxykinase (3). All these inhibitory effects are due to suppression of the activation of HIF-1α by CO, which binds noncovalently to ferrous heme groups (10).

The evidence summarized above speaks for a central role of heme-proteins in the form of 1) a mitochondrial complex in cytochrome oxidase and 2) a cytochrome b-like NAD(P)H oxidase on the plasma membrane.

SUMMARY OF MODEL OF OXYGEN SENSING AND RESPONSE

A model of oxygen sensing and responses in all mammalian cells is presented in Fig. 1. An exponential increase of response with decreasing PO2 below 30 Torr is seen in cytosolic mitochondria and also in membrane-bound NAD(P)H oxidase. The acute response follows from a cascade of reactions, which includes a cytosolic calcium rise, either from calcium stores and/or from entry from extracellular sources through the voltage-sensitive calcium channels. This may result from a decrease in outward potassium current through the plasma membrane. The same response could be initiated by CO. Another model (not shown here) suggests that hypoxia, by inhibiting cytochrome oxidase, causes the electron flow to back up, leading to mitochondrial ROS generation (see Ref. 20). However, this goes against a decrease in ROS generation due to hypoxia (1).

Chronic hypoxia, with the same initial responses, eventually inhibits the proteasome, which normally degrades HIF-1α. This reacts with preexisting HIF-β, producing a heterodimer called HIF-1, which then translates into the nucleus, inducing a battery of genes involved in the global response to hypoxia, including anaerobic metabolism, angiogenesis, and chemoreceptor responses, to restore a normal level of tissue oxygen and to assist cellular survival (20). The chronic response to hypoxia is inhibited by CO, acting at the plasma membrane level (see Fig. 1).

Figure 1 is merely a cartoon, and there are many gaps in the scheme. For example, hypoxic induction can still occur in cells deficient in HIF-1β and the genome of the yeast Saccharomyces cerevisiae lacks HIF-1 homo-

logue. Also, there are indications that some other factors, upstream of HIF-1α, must play a role (10).

LESSONS FROM HISTORY

Historically, many of the scientists of the past were as great as the scientists today. These scientists made mistakes in scientific judgement in the past, and similar mistakes are being made today at a higher level of knowledge, which cannot be avoided. That is a lesson from history.

I thank Dr. Robert E. Forster II for careful editing of the manuscript and for valuable advice. Figure 1 was produced by Drs. Arijit Roy and Charmaine Razanov. The manuscript was typed by Mary Pili. I am grateful for their help.

This work was supported in part by National Heart, Lung, and Blood Institute Grant R37-HL-43413-10. This work was written in sabbatical, partly at the Institute for Physiology, Ruhr University at Bochum, Germany, as a Humboldt Research Awardee. The author regrets that many important contributions could not be cited directly due to the brevity of this mini-review.

Address for reprint requests and other correspondence: S. Lahiri, Dept. of Physiology, Univ. of Pennsylvania Medical Center, Philadelphia, PA 19104-6085 (E-mail: slahiri@mail.med.upenn.edu).

REFERENCES

18. Ramsey WL and Wilson DF. Tissue capacity for mitochondrial oxidative phosphorylation and its adaptation to stress. In: Hand-


