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H_2O_2 Sensors of Lungs and Blood Vessels and Their Role in the Antioxidant Defense of the Body

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Abstract—This paper considers the composition and function of sensory systems monitoring H_2O_2 level by the lung neuroepithelial cells and carotid bodies. These systems are localized in the plasma membrane of the corresponding cells and are composed of O_2^--generating NADPH-oxidase and an H_2O_2-activated K^+ channel. This complex structure of the H_2O_2 sensors is probably due to their function in antioxidant defense. By means of these sensors, an increase in the H_2O_2 level in lung or blood results in a decrease in lung ventilation and constriction of blood vessels. This action lowers the O_2 flux to the tissues and, hence, intracellular $[O_2]$. The $[O_2]$ decrease, in turn, inhibits intracellular generation of reactive oxygen species. The possible roles of such systems under normal conditions (e.g., the effect of O_2^- in air) and in some pathologies (e.g., pneumonia) is discussed.

Key words: reactive oxygen species, H_2O_2 sensor, neuroepithelial cells, lung, carotid bodies

I am very much obliged to Sergei Eugenievich Severin. I had a chance to take a course of his bright lectures on the biochemistry and gases of blood, he was the advisor of my bachelor, master, and Ph.D. studies and then—just a wise adviser and friend who was always ready to help. It was Sergei Eugenievich who attracted my attention to the issue of the biological functions of oxygen in 1955. This problem became one of the main topics of my biochemical studies.

It is obvious that oxygen simultaneously performs several functions essential for aerobic life. It plays the role of terminal electron acceptor for the respiratory chain that is the major energy-providing mechanism for respiring cells. Moreover, O_2 is a substrate of oxygenases as well as of oxidases alternative to cytochrome oxidase of the respiratory chain. Some of these oxidases produce reactive oxygen species (ROS), namely O_2^- and H_2O_2, instead of the inert H_2O that is formed by cytochrome oxidase. ROS can be used by the organism as a tool to attack pathogens or as a signal. In the majority of cases, this is the signal for self-elimination of organelles, cells, organs, or even the entire organism. ROS are also formed nonenzymatically by "parasitic" chemical reactions of one-electron reduction of O_2 by the respiratory chain electron carriers and some other natural reductants [1-3].

It is noteworthy that H_2O_2 can be reduced by Fe^{2+} and Cu^+ to extremely dangerous hydroxyl radical ($OH^\cdot$), which is able to oxidize almost all cellular compounds including DNA. The high toxicity of ROS is due especially to $OH^\cdot$.

Higher organisms possess a multilevel system of anti-ROS defense. The first line of this system is to decrease the intracellular $[O_2]$ to a level still saturating cytochrome oxidase but insufficient for non-enzymatic ROS formation. One of the great achievements of evolution of aerobic life was the invention of cytochrome oxidase, an enzyme able to reduce O_2 at a high rate at O_2 levels even 100-fold lower than that in water under normal atmospheric pressure. As to ROS, their nonenzymatic formation parallels the decrease in $[O_2]$ according to the mass action law. This is why a decrease in intracellular $[O_2]$ over wide limits does not affect the cytochrome oxidase reaction but strongly inhibits nonenzymatic one-electron reduction of oxygen [1-4].

The strategy of higher organisms is that the rate of O_2 delivery to a tissue, being high in the state of active work, decreases dramatically during rest. This effect is achieved first of all by means of a decrease in lung ventilation and constriction of blood vessels at the work-to-rest transition.

It should be emphasized that reactive oxygen species, rather than O_2 per se, are dangerous. Therefore, it would be desirable for organisms to have a sensor monitoring the level of $OH^\cdot$, the most aggressive ROS. However, this is hardly possible since $OH^\cdot$, in fact, is too aggressive. On the way to the active site of the sensor, it would be discharged, spoiling thereby any cellular component includ-

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ing the hypothetical $OH^{\cdot}$ sensor itself. On the other hand, it would be much easier to monitor the level of H_2O_2, a direct precursor of $OH^{\cdot}$ in the chain of ROS interconversion reactions.

There are some indications that mammals possess at least two H_2O_2 sensors. One is located in cells of the lung neuroepithelial bodies, being responsible for constriction of the lung airways when the H_2O_2 level rises [5-7]. The other performs the same function in the blood vessels, being found in cells of the carotid body [8-10].

The two H_2O_2 sensors have very similar mechanisms as shown in the figure. They are composed of two independent protein systems, one H_2O_2-forming and another responding to H_2O_2. H_2O_2 is formed by an NADPH-oxidase which is of the same type as that found in the plasma membrane of phagocytes, where this enzyme forms O_2^- to suppress pathogens (for review, see [11]). The enzyme oxidizes intracellular NADPH, transporting electrons through the membrane to its outer surface (FAD and special two-heme cytochrome *b* are involved). Here one-electron reduction of O_2 to O_2^- occurs. Two O_2^- molecules dismutate to form O_2 and H_2O_2. The latter interacts with the outer part of a K^+ channel protein located in the plasma membrane. As a result, the channel is stabilized in its open conformation. If the O_2 concentration drops, the rate of the NADPH-oxidase reaction decreases, $[H_2O_2]$ decreases, the K^+ channel closes, the membrane potential on plasma membrane decreases, and the cell is excited. The excitation gives rise to release of intracellular serotonin to the extracellular medium. Serotonin operates as a mediator of opening of the lung airways (it is significant that the neuroepithelial cells are located in places of branching of these airways). This situation is typical for periods of active work when mitochondrial cytochrome oxidase consumes large amounts of oxygen:

Rest-to-work transition $\rightarrow$

$\rightarrow O_2$ consumption $\uparrow \rightarrow [O_2] \downarrow \rightarrow$

$\rightarrow [H_2O_2] \downarrow \rightarrow K^+$-channel $\downarrow \rightarrow$

$\rightarrow \Delta\Psi$ on plasma membrane $\downarrow \rightarrow$

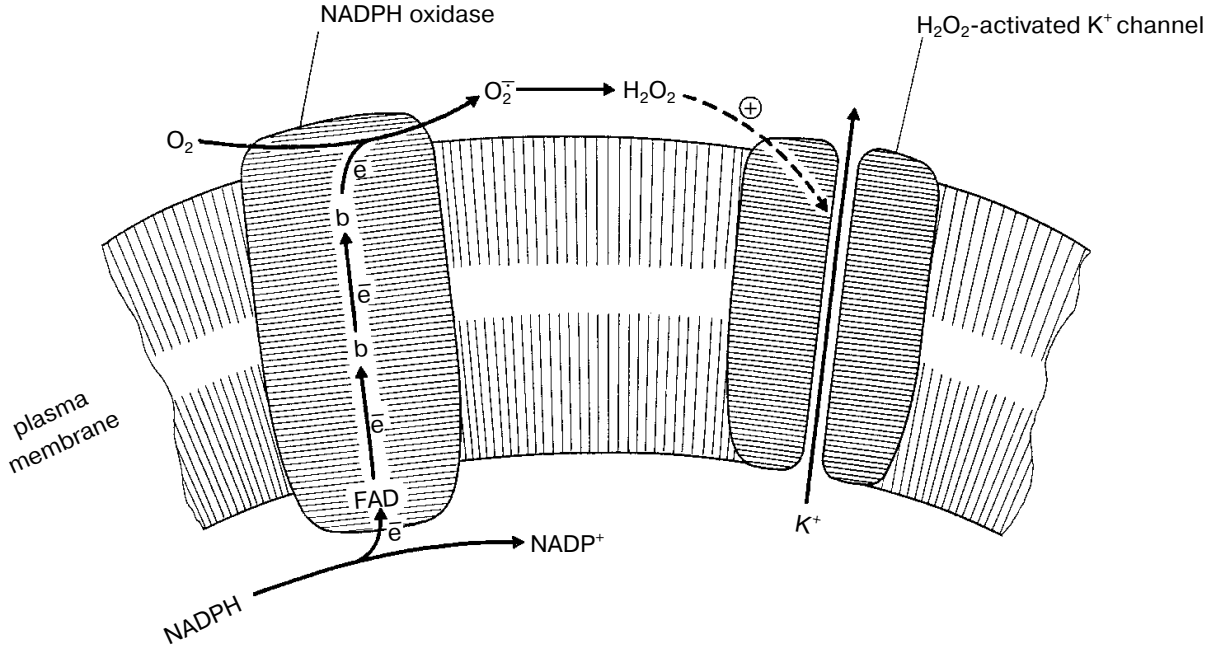
$\rightarrow$ serotonin release $\uparrow \rightarrow$

$\rightarrow$ opening of lung airways

The work-to-rest transition results in a decrease of the O_2 consumption in cells, a rise in the blood O_2 concentration, and consequent lowering of O_2 diffusion from the lungs to the blood. As a result, $[O_2]$ outside the neuroepithelial cells rises, NADPH oxidase is activated, $[H_2O_2]$ increases, K^+ channel opens, and serotonin is not released. The final event will be a decrease in the O_2 supply to the body due to a constriction of airways [8].

Similar events occur in the carotid cells. The only difference is that they release catecholamines instead of serotonin, causing dilatation of the blood vessels.

It is generally assumed that the lung neuroepithelial cells as well as carotid cells are O_2 sensors [8]. From this point of view, however, it is difficult to understand why



H_2O_2 sensor in the plasma membrane of the lung neuroepithelial and carotid cells [11]

the O_2 sensors of animals are organized in such a complex manner. It is known that the O_2 sensors are already inherent in bacteria, where they are much simpler than in animals and are competent in $[O_2]$ monitoring by means of a direct O_2 binding[1].

These relationships might be explained by suggesting that the major function of the sensors in neuroepithelial and carotid cells is that of the antioxidant defense of the organism. The very fact that it is $[H_2O_2]$ rather than $[O_2]$ that is monitored by these sensors allows the organism to effectively perform such a function. The described organization of the sensors causes constriction of the airways and the blood vessels due to an increase in $[H_2O_2]$ independently of the reasons causing this increase. The reasons may be not only elevation of $[O_2]$ because of a decrease in O_2 consumption in the tissues, but also activation of H_2O_2 production or inhibition of the H_2O_2 decomposition. This means that any damage to the antioxidant system of the body will actuate such an effective defense mechanism as a decrease in the O_2 supply to tissues and cells. Such a response would be impossible if in the above-mentioned sensory cells a simple bacterial-type O_2 sensor would be employed.

Quite recently, Weintraub and coworkers [16] reported that H_2O_2 activates an $O_2^{\cdot-}$-generating NAD(P)H oxidase in a non-phagocytic cell type of vascular origin (smooth muscle cells and fibroblasts). This means that production of H_2O_2 by, say, carotid cells can initiate a feed-forward mechanism amplifying the H_2O_2 signal. It is quite obvious that such a cascade may strongly reinforce the ability of ROS to down-regulate the O_2 delivery to the tissues.

The concept described above can explain a number of physiological and pathological phenomena. For example, bronchospasms in the case of pneumonia may be a consequence of an increased $O_2^{\cdot-}$ production by the phagocyte NADPH oxidase in the inflamed regions [11], an event erroneously interpreted by the organism as a signal of oxygen danger. The same situation may take place as a result of a viral infection in lungs due to activation of xanthine oxidase. As reported by Maeda and coworkers [17-19], the influenza virus causes strong (by 2-3 orders of magnitude) activation in lungs of xanthine oxidase, an enzyme forming $O_2^{\cdot-}$ and H_2O_2 from O_2 (concerning the possible significance of this effect for suppression of the viral infection, see [20]).

It seems possible that $O_2^{\cdot-}$ in the air (so-called negative aeroions) may regulate the work of the lungs. An increase in $[O_2^{\cdot-}]$ in the consumed air may be interpreted as a signal to decrease lung ventilation. Respectively, a decrease in $[O_2^{\cdot-}]$ will lead to hyperventilation, tissue $[O_2]$ increase, stimulation of ROS production, and, as a consequence, acceleration of ageing [2].

In this context, it should be noted that one of the most aggressive types of cancer, small cell lung carcinoma, represents, in fact, a result of malignant transformation of the lung neuroepithelial cells. These tumor cells still produce the same neuromediators [21, 22] and contain both NADPH oxidase and the H_2O_2-stimulated K^+ channels [8]. Even more, malignant transformation was found to be oxygen-dependent [23].

It seems possible that the favorable effects of $O_2^{\cdot-}$-generating devices (see, e.g., [24, 25]) is also mediated by some H_2O_2 sensor(s). As shown by Goldstein and coworker [24], mice and rats die when kept under $O_2^{\cdot-}$-free conditions for 16 and 23 days, respectively. Most probably, the death is a consequence of deregulation of some functions of vital importance occurring due to the absence of signals from $O_2^{\cdot-}$- and/or H_2O_2-sensors normally reporting about the level of these reactive oxygen species in airways. These signals might be produced by either lung neuroepithelial bodies (see above) or the so-called vomeronasal organ [26, 27]. There are indications that in large cities the air $[O_2^{\cdot-}]$ is strongly reduced due to antioxidant actions of products of decomposition of rubber and some other polymers [28, 29]. The air $O_2^{\cdot-}$ deficiency could be compensated by artificial $O_2^{\cdot-}$-generators. However, here we should be very careful since hyperproduction of $O_2^{\cdot-}$ can lead to catastrophic consequences due to constriction of the lung airways. This is why the use of $O_2^{\cdot-}$ generators should be considered only after detailed investigation of their effects on lung function. In any case, it is very probable that air $O_2^{\cdot-}$ monitoring should be useful to improve conditions of existence of humans in the modern world.

[1] One of them was quite recently described by Alam and coworkers in *Halobacterium salinarium* and *Bacillus subtilis*. This is a single protein composed of two domains. The first (175 amino acid residues) is homologous to the animal myoglobin, whereas the second (amino acids 222-489) is very similar to the bacterial methyl-accepting proteins taking part in chemotaxis. They assume that the O_2 binding by the first domain results in a conformational change transmitted to the second domain participating in transduction of the signal to the bacterial flagellum [12]. There are some reasons to suggest that heme-containing O_2 sensors that bind O_2 without its subsequent reduction operate also in animals, but their role consists in regulation of some events at the level of the cell rather than the organism (for review, see [13-15]).

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