Physiology of Aging

Selected Contribution: Carotid body as a model for aging studies: is there a link between oxygen and aging?

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Aging is characterized by a reduction in general homeostatic adaptation to metabolic requirements, a decrease in oxygen supply to tissues and $P_{O_2}$ (3, 12), and a reduction in the activity of several metabolic factors and enzymes such as endothelial nitric oxide synthase (eNOS) (7, 23). Indeed, eNOS is important for peripheral vasodilation response to hypoxia.

Chronic hypoxia per se promotes a remodeling of the structure and function of cardiorespiratory system, the brain, the kidneys, liver, and muscle (14, 27). At altitude, hypocapnia, consequent to hyperventilation, plays a great role in adaptation through respiratory alkalosis that interferes with cell growth and metabolism. During aging, a reduction in homeostatic processes involving tissues and organs, such as heart, kidneys, and liver (2, 29), could include also CB cell adaptation processes so affecting oxygen supply to tissues.

In general, acute hypoxia increases firing by chemosensory fibers (6). This is less evident during aging as well as during long-lasting hypoxia in young subjects. On the other hand, chronic hypoxia leads to enlargement and hypertrophy of type I cells ($\times 4$) and enhancement of catecholamine content ($\times 15$) with a blunted respiratory response. These phenomena seem to suggest that chronic hypoxia acts on factors involved with cell growth and neurotransmitter release. In conditions of hyperoxia followed by hypoxia, the response to hypoxia is attenuated, whereas that to carbon dioxide remains unaltered (17), suggesting that the sensitive mechanisms for carbon dioxide and oxygen are not necessarily the same. The ventilatory response to hypoxia is characterized by increase in ventilation based on the degree of hypoxia. This response is attenuated with aging, and it is related to the age-dependent structure modifications and functions, including the basal reduction of oxygen requirements (10, 11).

Input and output functions are fundamental for the ventilatory response and are related to afferent nerves, efferent nerves, respiratory centers, intercostal muscles, and phrenic nerve. During aging, a reduction in nerve conductivity occurs, i.e., the maximum number of impulses per minute decreases, and so does sensi-
tivity by peripheral receptors, all resulting in a reduction in homeostatic capacity and higher latency in the adaptation responses (9).

Generally cell growth, differentiation, aging, and death are related to a series of factors including oxygen consumption, intracellular pH, free radical production, and oxygen supply to tissues. All these factors depend on CB function. Therefore, CB is an excellent model for studying aging processes due to its high blood flow and metabolism.

METHODS

Four groups of Wistar rats 200–250 g were used according to the guidelines of the Declaration of Helsinki. Two groups of six rats each (age-matched 2 and 24 mo old) were kept in room air (21% oxygen) and served as control; the other two groups (age-matched 2 and 24 mo old) were kept in a Plexiglas chamber for 12 days in chronic hypoxia (10–12% inspired oxygen). Chamber temperature and carbon dioxide were kept in physiological ranges. The rats were anesthetized with 30 mg/kg ip Nembutal, carotid bifurcation was exposed, and the rats were perfused and superfused with glutaraldehyde 2.5% in phosphate buffer, pH 7.4, 320 mosM. CBs were dehydrated in ethanol and embedded in hypoxy resin. Ultrathin sections were cut and mounted on 200-mesh copper grid. Randomly selected fields were used for electron microscopy. Positive prints were enlarged to obtain final magnification of 26,000–56,000. The quantification of the results was made by stereomicroscopic measurements using the Bioquant system interfaced through a digitizing tablet to a microcomputer. Visual examination of many photomicrographs was performed.

Light microscope determinations of CB volume were carried out in the same way described by Edwards et al. (8).

Statistical analysis. The values obtained from 12–15 electron micrographs were averaged for any given CB. Mean values were calculated from the morphometric measurements of the cell parameters. The unit of measure for type I cell cytoplasmic area, nuclear area, and single mitochondria area are expressed as square pixels. Statistical comparison between the young group under hypoxic treatment and the corresponding age-matched group, relatively to each morphometric variable under investigation, was performed by use of t-test (for unpaired data) with values of $P < 0.05$ considered significant. The same analysis was performed for the old group. The experimental data for each group were also analyzed by one-way ANOVA with the same $P$ value chosen as for the $t$-test considered significant. The hypoxic response in the young and old groups was evaluated with the same statistical procedures.

RESULTS

Optical microscopy. The normal lobular structure of a young CB is shown in Fig. 1A. Figure 1C depicts hypoxic young CB.

CB seems to be bigger with an increase in glomoid tissue cells. Figure 1B shows an aged rat with an increase in extracellular matrix, and Fig. 1D represents the old CB after hypoxia. The number of CB cells are reduced in aging. CB is enlarged due to hyperplasia of CB cells both in young and aged and it represents a hypertrophic response to hypoxia. This response is preserved during aging but is less evident.

DISCUSSION

The aged CB shows an increase in extracellular matrix and a reduction in number and volume of type I cells compared with young CB. Chronic hypoxia reduces the volume and density of mitochondria, which represents an adaptive response to hypoxia as a consequence of the reduced oxygen consumption by the mitochondrion itself. This phenomenon seems to operate also during aging as shown by the reduced number...
and volume of mitochondria in the aged CB. Therefore, hypoxia and aging seem to share some type of link at different cell sites. Hypoxia per se modulates mitochondrion activity, influencing oxygen consumption. In turn, oxygen consumption affects gene expression and aging processes. Furthermore, prolonged hypoxia and aging have both been shown to cause accumulation of lipofuscin in muscles (1, 19).

During chronic hypoxia, CB hypertrophy is less evident in aged CB than in young CB, which is probably related to a reduced release in growth factors during aging. Furthermore, such a reduction in hypertrophy could be due to general protein structural changes occurring during aging. Such protein changes could involve also a plasma hemoprotein, which has been suggested in the chromophore theory of chemoreception. Indeed, such a protein has been claimed to be an oxygen-, carbon monoxide-, and cobalt-sensitive site.

Table 1. *Morphometric variables of type I cells in young rat carotid body after chronic hypoxia*

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<th>Young Normoxic</th>
<th>Young Hypoxic</th>
<th>P</th>
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<tbody>
<tr>
<td>Type I cells cytoplasmic area</td>
<td>19,004 ± 132</td>
<td>25,418 ± 1,316</td>
<td>0.0007</td>
</tr>
<tr>
<td>Type I cells nuclear area</td>
<td>12,714 ± 813</td>
<td>11,346 ± 1,013</td>
<td>0.3170</td>
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<tr>
<td>Number of mitochondria per type I cell</td>
<td>28.6 ± 3.1</td>
<td>23.1 ± 2.7</td>
<td>0.2106</td>
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<tr>
<td>Single mitochondrion area</td>
<td>71.3 ± 1.6</td>
<td>54.9 ± 3.3</td>
<td>0.0012</td>
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Values are means ± SE in pixel²; n = 6 rats for each experimental condition. A P value <0.05 was considered significant.

Such effects on the hemoprotein structure could contribute to the reduced response to hypoxia by the aged CB. Hypoxia has been shown to induce modifications in the CB, including the changes in size and weight, which reflect the changes involving the various components of the CB itself, including CB connective tissue, blood vessels, and mitochondria-to-cytoplasm ratio. Such changes seem similar to those observed in the aged CB, as shown by the reduction in mitochonodrion volume. A similar mechanism could be operating during CB arteriosclerosis (13). Indeed, arteriosclerosis per se induces a state of hypoxia by stimulating extracellular matrix increase, thus increasing the distance between cells and blood vessels, which results in a reduced oxygen diffusion gradient.

On the other hand, it seems important to consider the fact that oxygen is toxic. Indeed, at high concentrations it can interact with oxygen-sensitive molecules probably located inside mitochondria, and this latter could represent the prime oxygen-sensitive site. First, life span and maximal oxygen uptake are correlated with the volume and function of mitochondria (21). Moreover, oxygen consumption is critically involved with capillary network, cell metabolism, and peripheral tissue oxygen (26). Finally, it has been previously argued that chronic hypoxia increases mitochondria volume with loss of structure and function. Hyperoxia damages mitochondrial DNA, and this latter is more vulnerable than nuclear DNA, which is protected by histonic proteins. Furthermore, hyperoxia induces excessive free radical production with release of toxic species in the cell; in fact, a 48-h exposure of cells to an environment containing 80–100% oxygen promotes progressive loss of reproductive capacity, growth inhibition (24), mitochondrion impairment, and inactivation of enzymes containing sulfhydryl groups, e.g., flavoprotein (15). In animal experimental models, 100% oxygen results in death of all animals after 4–5 days of exposure (22). Dejours and Dejours (4) described a physiological denervation of CB after hyperoxia. CB denervation could be linked to free radical effects on plasma membrane. Finally, our previous results show that hyperoxia damage is more pronounced in young than in old rat CB, and it could be safely hypothesized that oxygen-sensitive mechanisms decrease with age (5).
It is worth noting that mitochondrion plasticity and adaptation related to oxygen availability are surprising. Tissues and cells seem to be more adapted to hypoxia than to hyperoxia, and they seem to be more capable of surviving in hypoxia than in hyperoxia environment (20). The reduction of oxygen consumption during hyperoxia seems to work as a self-adapting mechanism aimed at protecting cells. This is somewhat similar to the increasingly lowering of the degree of oxygen consumption throughout life and the reduction of maximum oxygen consumption during aging. In fact, we have previously shown that the aged CB had a lower damage due to hyperoxia compared with young CB. It could be safely argued that such a lowering could represent some sort of insensitivity to hyperoxia by the aged CB. Moreover, aging is associated with alterations in oxidant-antioxidant balance (25, 28). Thus it seems as if throughout life cells and tissues became insensitive or adapted to oxygen and to free radicals. The link between oxygen consumption and aging regarding hypoxia and hyperoxia should give an idea of the complexity of metabolic needs.

In conclusion, CB represents an experimental model adequate for studying aging processes because of its high blood flow and metabolism related to the oxygen-sensitive mechanisms; thus it serves as a means to understand whether modifications in oxygen supply to cells through hypoxia and/or hyperoxia are capable of modulating the aging process.

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REFERENCES