ULTRASTRUCTURAL DEGRADATION OF THE CAROTID BODY IN THE AGED RAT: IS THERE A ROLE FOR ATHEROSCLEROSIS IN THE MAIN CAROTID ARTERIES?

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In this study we examined the potential role of atherosclerosis in the main arteries supplying blood to the carotid body in the organ’s morphological degradation with age. We addressed this issue by comparing the ultrastructural picture of carotid bodies and of fragments of the carotid artery bifurcation in two age-extreme groups of rats: young - 3 months old and senescent - 24 months old. Tissues were excised under surgical anesthesia, fixed in aldehydes, and processed for transmission electron microscopy. We found that the old carotid body parenchyma exhibited profound degenerative changes. Chemoreceptor cells were at various stages of atrophy, ranging from swollen mitochondria and fewer secretory vesicles to dark dehydrated cells. In contrast, the senescent carotid artery bifurcation was little different from that in young rats. Particularly, endothelial cells were in perfect condition. There were some changes in deeper arterial wall layers such as breaks in the continuity of elastic bands or a subtly different phenotype of smooth muscle cells. No foam cells or calcium build-ups were found in the arterial walls. Such changes correspond to the process of arterial wall stiffening in old age rather than to the outright atherosclerosis. Lack of atherosclerosis in the common carotid arteries, which could hamper blood flow, argues against its playing a role in the morphological age-changes in the carotid bodies.

Key words: aging, atherosclerosis, carotid artery bifurcation, carotid body, degeneration, ultrastructure
INTRODUCTION

Carotid body is a paired organ localized to the bifurcation of the common carotid artery. These minute organs have a profound physiological significance, as they generate ventilatory hyperventilation in response to deficit of oxygen in inspired air (1). There are human studies showing that carotid body function, expressed as hypoxic ventilatory sensitivity, declines with age (2, 3), which would agree with the common intuit. However, the issue is contentious, as others show sustained ventilatory responses to hypoxia in old age (4, 5). The nature of a possible functional carotid body decline is unclear, but one mechanism could be the age-related processes in carotid body parenchyma. Indeed, in previous work we found signs of possible tissue degeneration in old rat carotid body (5, 6). Age-changes in the carotid bodies have also been observed in a human study, in which the organs were removed post mortem (7). In that study profound atherosclerosis also was noted in the region of the common carotid artery bifurcation through which blood supply to the organ flows. The question, therefore, arises of whether age-changes in the carotid body would be due to hampered blood supply to, and secondary to it chronic hypoxia, in the organ or due rather to tissue aging per se. In the present study we addressed this issue by comparing the ultrastructure of carotid body parenchyma and carotid artery bifurcation wall fragments in two age-extreme, young and senescent, groups of rats; the species that is known, as opposed to humans, to be fairly resistant to developing typical atherosclerosis (8, 9).

MATERIAL AND METHODS

The study was approved by a local Ethics Committee (Permits no. 109 and 137). Ten male, Wistar rats, divided into 2 groups: young -3 months old and old - >24 months old were used for the study. The animals were raised in the institutional animal house, maintained at controlled temperature of 21°C in a 12-h day/night cycle, fed with commercial rodent chow, and had water ad libitum. At the day of the experiment, the animals were anesthetized with α-chloralose and urethane (15 mg and 75 mg/100 g for young and 10 mg and 50 mg/100 g body weight for senescent) administered intraperitoneally. The carotid bodies were exposed surgically, and then the animals were euthanized by perfusion with an aldehyde mixture (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M cacodylate buffer, pH 7.4) through the heart. The carotid bodies and wall fragments of the carotid artery bifurcations were rapidly dissected and postfixied in the same mixture. The material was incubated in a 2% OsO₄ solution, dehydrated in a series of increasing ethanol concentrations and propylene oxide, and finally was immersed in Spurr resin. Ultrathin sections (50nm) were cut, mounted on copper grids, and examined under a JEM 1200EX transmission electron microscope (Jeol; Tokyo, Japan).
RESULTS

Carotid body ultrastructure

Fig. 1A shows a typical ultrastructural appearance of a young carotid body. Clusters of chemoreceptor cells with adjacent nerve fibers are surrounded by connective tissue and capillary vessels. The chemoreceptor cells have oval nuclei with regularly scattered chromatin. The cytoplasm of these cells contains numerous mitochondria, well-developed Golgi apparatus, and abundant dense-core vesicles. By contrast, parenchyma of a senescent carotid body shows a number of degenerative alterations (Fig. 1B). The most striking feature is a considerably thicker layer of connective tissue that apparently causes a squeeze on the chemoreceptor cells whose nuclei become out of shape. There are swollen mitochondria with distorted or lost internal cristae, the volume of the Golgi is reduced, and there are fewer dense-core vesicles. Occasionally, in the old carotid bodies we observed dark, dehydrated cells with shrunk cytoplasm, and nuclei with irregular nuclear heterochromatin; the picture reminiscent of apoptotic-like cell death. However, the ultrastructural picture of old carotid bodies was variable. Interspersed with altered chemoreceptor cells, there were isles of near-normal looking cells.

Fig. 1. Photomicrographs of young (A) and senescent (B) rat carotid bodies. CC - chemoreceptor cells, C - capillaries, Co - collagen, EC - endothelial cells, NF - nerve fiber. Magnification - 3k.
Carotid artery ultrastructure

Fig. 2A presents a typical cross-sectional appearance of a fragment of a young carotid artery wall. The luminal surface is covered by endothelial cells whose cytoplasm is rich in organelles and contains shapely formed nuclei. The ultrastructure of a senescent carotid artery wall is surprisingly well preserved (Fig. 2B). The endothelial cells and junctions between them appear in a perfect condition. There were some changes observed in deeper arterial wall layers, such as breaks in the continuity of elastic bands, proliferation of collagen fibrils, and a subtly different phenotype of smooth muscle cells. These changes were modest. There were no foam cells or calcium build-ups in the vessel wall, which could be described as typical for atherosclerosis.

DISCUSSION

The study demonstrates that there were rather profound morphological alterations in carotid body parenchyma in senescent rats at a time when there were no major alterations visible in carotid artery wall. Since carotid body blood, and thus oxygen,
supply comes from the region of carotid artery bifurcation, the obvious inference is that the age-changes in old carotid bodies would be due to reasons other than physically hampered blood flow through the major arteries to the organ.

The region of carotid artery bifurcation is known to be most sensitive to the development of atherosclerosis due to a number of local factors, such as variations in flow velocity, turbulence, flow separation, and increased artery wall stress (10). These conditions facilitate release of platelet-derived growth factors that induce cell proliferation, inflammation, intimal thickening, and plaque formation in the arterial wall (11). Such changes are well known to hinder blood flow through the carotid artery in humans and understandably could also limit blood supply to the nearby carotid bodies with all the functional consequences for the organ. In a human study, Lowe et al (7) have found both age-changes in post mortem morphologically studied carotid bodies and massive atherosclerotic build-ups in the bifurcation area in senescent persons. The authors suggested that the atherosclerotic status of the carotid arteries could reflect on the morphological status of the carotid bodies.

In the present study we did not corroborate the concurrence of atherosclerosis in the carotid bifurcation and carotid body morphological degradation in the senescent rat. Age-dependent, mild morphological remodeling of the arterial wall concerned the components of its deeper layers, such as elastic fibers or smooth muscle cells. Elastic fibers gradually lose elasticity with age becoming more susceptible to elastolytic enzymes (12). Such changes, not obstructing the vessel lumen, correspond to modest arterial wall stiffening normally developing in old age rather than to atherosclerosis, as there were no foam cells, fatty build-up, and other typical atherosclerotic alterations involving endothelial cells. Relative resistance of the rat carotid artery to typical atherosclerosis, we observed, is in accordance with other data from the literature (8, 9). At the same time, parenchyma of the old carotid body was distinctly affected. Extensive proliferation of glomic connective tissue could make the diffusion pathway for oxygen from capillaries to chemoreceptor cells longer, which would produce stagnant hypoxia with all the untoward consequences for carotid body morphology.

The issue of carotid body morphological aging and its functional correlates remains unresolved. The limited knowledge on the issue stems likely from the impossibility to conduct prospective morphological studies of the organ. Studies of carotid body function are often, and in humans exclusively, indirect, as the function is expressed by the hypoxic ventilatory response (HVR). The results of such studies are mixed. Some authors show a dampening of HVR in old age (2, 3), while others show no change in it (4, 5). The animal studies on the subject are scarce and their results are hardly conclusive either. Schlenker and Goldman (13) have shown that ventilation attains a higher level in response to hypoxia in 12 months old than in 24 months old Wistar rats, but hypoxic sensitivity, as measured from the slope of ventilation on oxygen tension, remained unchanged with age. Fukuda (14) has shown that HVR in 20 months old rats is attenuated compared with that in 1.5 months old rats. The author ascribes the attenuation to
lower oxygen consumption rather than inefficient carotid body function in old age. Therefore, carotid body function in old age remains unsettled and apart from its plausible dependence on carotid body morphology, it also may depend on the species and compensatory abilities to uphold hypoxic ventilation.

In conclusion, lack of typical atherosclerosis, which could hamper blood flow, in the common carotid arteries in senescent rats argues against its playing role in the morphological age-changes in the carotid bodies. The exact determinants of carotid body degradation in old age and its functional correlates could not be discerned in our study and would require alternative study designs.

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REFERENCES


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