MORPHOLOGICAL AND NEW NEUROCHEMICAL ASPECTS OF THE MAMMALIAN CAROTID BODY

N. Lazarov1,2*, D. Atanasova2,3

1Department of Anatomy and Histology, Medical University of Sofia, Sofia, Bulgaria
2Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
3Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

ABSTRACT
The carotid body (CB) is a polymodal chemosensory organ that plays an essential role in initiating respiratory and cardiovascular adjustments to maintain blood gas homeostasis. It is strategically located at the carotid bifurcation. The CB works in concert with the apposing afferent nerve endings of the petrosal ganglion (PG) cells and together they form a functional unit, the CB chemosensory system. The organ consists of small clusters called glomeruli composed of two cell types, glomus and sustentacular cells, interspersed by blood vessels and nerve bundles, and separated by connective tissue. During chemotransduction glomus cells release a variety of neurotransmitters which activate chemoafferent nerve endings of PG neurons. Much of the available evidence suggests that the CB dysfunction and altered oxygen homeostasis are involved in the pathophysiology of several diseases including systemic hypertension. Our recent data show that in glomus cells of hypertensive animals the production of nitric oxide is impaired and components of the neurotrophic signaling system display enhanced expression. These results suggest that a heightened chemosensory discharge may contribute to sympathetic hyperactivity leading to hypertension. Knowledge of the morphofunctional and neurochemical aspects of the CB would improve our current understanding of respiratory and cardiovascular homeostasis in health and disease.

Key words: chemoreception, glomus cells, hypertension, neurotrophic factors, nitric oxide, spontaneously hypertensive rats

INTRODUCTION
The carotid body (CB) is a small paired neural crest-derived structure bilaterally located at the common carotid artery bifurcation (1). This location is strategic for monitoring the arterial blood levels of oxygen and carbon dioxide as well as the hydrogen ion concentration (pH). Their significant changes are detected by the chemoreceptive cells in the organ which depolarize and release a neurotransmitter that stimulates apposed chemoafferent nerve endings of the petrosal ganglion (PG) cells, and signals brain centers to initiate corrective respiratory and cardiovascular reflexes to restore homeostasis (2, 3).

The CB has recently been implicated in the pathophysiology of various cardiovascular diseases, including certain forms of hypertension (4). Thus, a better understanding of the CB role in sympathetic-mediated diseases such as systemic hypertension would contribute to the development of new therapeutic strategies for the treatment of hypertensive patients.

GENERAL STRUCTURE AND NEUROCHEMISTRY OF MAMMALIAN CAROTID BODY
The CB is structurally complex and its basic plan is quite similar in all mammals. The CB parenchyma is organized in cell clusters called glomeruli, separated by septa of connective tissue, and composed of two juxtaposed neural crest-derived cell types: type I or glomus cells and type II or sustentacular cells (Figure 1). Being a neurovascular structure, the CB is...
highly vascularized (5) and densely innervated by both sensory and autonomic nerve fibers (6, 7). The neuron-like glomus cells, the principal CB cell type, contain numerous dense-cored vesicles storing and releasing various neurotransmitters. They are regarded as the chemosensory cells of the CB (2). The glial-like sustentacular cells are classically considered to be supporting cells which play a role of metabolic support, and in addition to this, they have recently been assumed to be the CB stem cells (8). Emerging evidence also suggests that sustentacular cells serve as paracrine modulators of CB chemoreception (9). Moreover, autonomic ganglion cells, both sympathetic and parasympathetic, are described in the CB (10) and they are present with varying location and number in most mammals among the different animal species and breeding strains (11).

**Figure 1.** The general structure of the carotid body (CB).

(A) Micrograph of a Nissl-stained section illustrates the strategic localization of the CB between the external carotid artery (ECA) and the internal carotid artery (ICA). (B) Representation of a CB glomerulus with van Gieson staining. The septa of collagen fibers of the glomic capsule with vascular connective tissue (CT) are stained in red. Also note the numerous blood vessels (BV) in the CB parenchyma. (C) A cell cluster consisting of groups of oval glomus cells (G) partially invested by elongated sustentacular cells (S). (D) H&E staining of a representative CB glomerulus indicating its innervation from the carotid sinus nerve (CSN). SCG, superior cervical ganglion. Scale bar = 100 µm.

Appropriate ion channels in the glomus cell membrane respond to stimuli in the arterial blood, primarily hypoxia, hypercapnia or acidosis, by activating the release of neurotransmitters. Amongst them, the biogenic amines, including acetylcholine, norepinephrine, dopamine and serotonin, comprise the largest group (12, 13). In the recent years, histamine has also been considered a putative transmitter in hypoxic chemosensitivity in rats and humans [14, 15]. Other neuroactive substances such as some neuropeptides (substance P, VIP and enkephalins) and the gaseous neuromessenger molecules nitric oxide and carbon monoxide also play a role in oxygen sensing in the mammalian CB as neuromodulators or second messengers, respectively (16, 17). In turn, these neurotransmitters contribute to the modulation of glomus cell function via autoreceptors.

**CAROTID BODY AND MECHANISMS OF HYPERTENSION**

It is generally accepted that essential hypertension is characterized by hypersensitivity to hypoxia, an increased sympathetic vasmotor tone and elevated catecholamine content. It has lately been revealed that the CB input plays a fundamental role in both the genesis and maintenance of hypertension (18) since heightened CB chemoreceptor drive elicits sympathetic hyperactivity, which is a common hallmark of
sympathetic-related diseases (19, 20). However, the triggers leading to increased CB sympathetic vasoconstrictor tone which underlines hypertension are not yet fully known.

A growing body of evidence suggests that nitric oxide (NO) increases the CB chemosensory activity, and the enhanced peripheral chemoreflex sensitivity contributes to sympathoexcitation and consequent pathology (18, 19). Our recent findings show that in the hypertensive state the expression of all three main NO synthase (NOS) isoforms appear to be changed in the rat CB. Specifically, we find that the neuronal NOS (nNOS) and endothelial NOS (eNOS) are statistically down-regulated while the levels of inducible NOS (iNOS) are up-regulated in the CB (Figure 2 and Figure 3). In fact, we observe nNOS-immunoreactive varicosities in close proximity to the CB cell clusters. It seems that the altered NO production modulates the chemosensory processing in hypertensive animals by its direct action on glomus cells and indirectly on the CB vasculature (21).

Previous research has revealed that the endothelial dysfunction in hypertension may not be associated only with an impaired NO metabolism but also with an increased production of angiotensin II (Ang) (reviewed in 22). Accordingly, our recent research has

Figure 2. Patterns of NOS immunohistochemical staining in the carotid body (CB) of normotensive Wistar rats (NWR) and spontaneously hypertensive rats (SHR).
(A) nNOS-immunostaining is present in thin varicosities around the immunonegative glomus cells. Occasional nNOS-containing cells are seen at the periphery of the normotensive CB. (B) eNOS-immunoreactive nerve fibers in the vicinity of immunonegative glomus and endothelial cells of the blood vessels in the CB of NWR. (C) A number of iNOS-immunoreactive neurons are found dispersed in the CB of control animals. (D) Sparse varicose nNOS-immunostained nerve fibers are observed in close proximity to clusters of glomus cells and are also associated with CB vasculature. eNOS-immunopositive varicosities and dot-like structures are visible in the vicinity of glomus and endothelial cells in the hypertensive CB (E). A subset of glomus cells and the walls of blood vessels show moderate-to-high intensity staining for iNOS in the hypertensive CB (F). Scale bars = 100 µm.

Figure 3. Statistical comparison of the immunostaining intensity for the three isoforms of NO in normotensive Wistar rats (NWR; brown boxes) and spontaneously hypertensive rats (SHR; green boxes). The nNOS (A) and eNOS (B) staining intensities were statistically decreased in the CB of hypertensive compared to normotensive animals. (C) Prominent increase in the iNOS immunostaining was observed in the CB of SHR. The boxes represent the second and third quartiles (25-75%), the line within the box represents the median, and lines outside the box represent the spread of values. p<0.001 in comparison with the NWR group.
demonstrated that the glomus cells are richly endowed with angiotensin AT1 receptors (23). Possibly, simultaneous activation of the renin-angiotensin system and the interaction of NO with Ang II may further potentiate the direct effects of NO on blood vessels.

Our studies have also demonstrated that the hypertensive CB shows structural plasticity and could enlarge without vasodilation (24). We believe that the structural alterations may be explained through the enhanced expression of trophic factors and their receptors, which act on CB cell populations in autocrine or paracrine ways. In particular, we find that the majority of glomus cells and a subset of sustentacular cells in spontaneously hypertensive rats (SHRs) intensely produce neurotrophic factors of the NGF and GDNF family and the corresponding receptors, and their expression levels are significantly increased in comparison with these in the normotensive CB (Figure 4). Moreover, we show that the hypertensive adaptation of the rat CB involves proliferation of sustentacular cells as well. It is likely that the glomus cells exert, via the entire cocktail of neurotrophins present in them, trophic and/or regulatory control on the adjacent cells in the CB. Based on both morphometrical and immunohistochemical analysis, we predict that the increased levels of these neurotrophins in this strain of rats are responsible for higher CB activity, leading to enhanced sympathetic tone that in turn may contribute to the development of hypertension. Such an idea coincides with the increased noradrenaline content in the hypertensive CB reported by others (25). Alternatively, but less likely, hypertension is capable of altering the expression profiles of neurotrophic factors in the CB which can result in morphological changes promoting survival of its cell populations under hypertensive conditions. Even so, these adaptive alterations are most probably affected by the associated overactive sympathetic activity in SHRs, as proposed by Kato et al. (25).

**Figure 4.** Immunohistochemical demonstration of neurotrophins and neurotrophin receptors in the normotensive (right column) and hypertensive (left column) carotid body (CB). Light photomicrographs showing that the cell clusters of spontaneously hypertensive rats exhibit immunoreactivity for NGF (A) and BDNF (E) and their receptors TrkA (C) and TrkB (G), respectively. Note that the immunostaining is observed in the cytoplasm of a large number of glomus cells and in a subset of sustentacular cells in the hypertensive CB and it is enhanced compared with that in the normotensive CB (B, D, F, H). Scale bars = 100 µm.
In conclusion, our study suggests that a heightened chemosensory discharge causes sympathetic neural activation which, along with the impaired NO synthesis and the elevated production of neurotrophic factors, could contribute to the development of hypertension. Last but not least, lessons from studies with hypertensive animal models indicate that NO and neurotrophins appear to be involved in the genesis of hypertension and its maintenance in the SHR, presumably through a chemoreceptor-related stimulation of the sympathetic activity.

ACKNOWLEDGEMENTS
The authors would like to thank Sabina Mitova for her excellent technical assistance.

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