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Mast Cells in the Rat Carotid Body

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The carotid body (CB) is the main peripheral arterial chemoreceptor in mammals that registers the oxygen and carbon dioxide levels in blood, and responds to their changes by adequately adapting the cardiovascular and respiratory homeostasis. The basic morphofunctional unit of the CB called 'glomerulus' consists of two juxtaposed cell types: chemosensory neuron-like type I or glomus cells, and type II or sustentacular cells, the latter being supporting glial-like cells. The purpose of this study was to determine the presence and distribution of mast cells in the rat CB by using staining techniques with Toluidine blue and Bismarck brown. In particular, the mast cells were predominantly located in the interlobular connective tissue of the CB and were closely associated with blood vessels, but they were not found within the cell clusters. A few were observed in a close association with the islands of cells, and they were related to the sustentacular cells. Thus, the mast cells are not directly associated with glomus cells and probably do not functionally determine chemosensory properties. It is likely that mast cells are involved in the regulation of the blood supply within the CB by acting on small blood vessels.

Key words: carotid body, mast cells, blood vessels, toluidine blue, Bismarck brown

Introduction

The carotid body (CB) is the peripheral arterial chemoreceptor that registers the levels of pO2, pCO2 and pH in the blood and responds to their changes by adequately adapting the cardiovascular and respiratory homeostasis. It is strategically located in the bifurcation region of each common carotid artery. The organ consists of "glomeruli" composed of two cell types, glomus and sustentacular cells, interspersed by blood vessels and nerve bundles, and separated by connective tissue.

Mast cells (MCs) have a widespread tissue distribution [4] and are considered to be multifunctional immune cells involved in health and several diseases. Due to their extensive tissue distribution and adaptability they are able to react to environmental changes by comunicating with other cells implicated in physiological and immunological responses.

MCs are plentiful in the CB of humans; their numbers vary in different animal species [10] and are not related to the CB histology. However, there is no difference in the number of MCs in the CBs of young patients with prominent glomus cells and adults with many supporting cells or age-related acellular fibrosis [10].

Chiocchio et al. [3] suggested that dopamine might be stored in the MCs of the cat CB and it might be a precursor of other catecholamines. In addition, there is evidence for serotonin-like immunoreactivity not only in the glomus cells but also in the MCs of the rat CB [7].

Therefore, we set as a goal of this study to provide morphological data on the presence and distribution of MCs in the rat CB using classical histological techniques.

Materials and Methods

The experiments were carried out on eight male Wistar rats, weighing 220 – 250 g. The study was performed in agreement with the European Communities Council Directive 2010/63/EU for the protection of animals used for scientific purposes. The experimental procedures have been approved by the Institutional Ethics Committee at the Institute of Neurobiology of the Bulgarian Academy of Sciences.

The animals were deeply anesthetized and transcardially perfused first with 0.05 M phosphate-buffered saline (PBS), pH 7.4, followed by 4% paraformaldehyde (PFA) in 0.01 M phosphate buffer (PB), pH 7.4. The carotid bifurcations were dissected out and postfixed in the same fixative overnight at 4°C. Thereafter, the tissues were embedded in paraffin and cut into 6 μm thick serial sections. Adjacent serial sections were mounted on glass slides coated with chrome alum-gelatin and processed for Hematoxylin and Eosin (H&E), Toluidine blue (TB) and Bismarck brown (BB) staining, respectively.

For the Toluidine blue staining samples were deparaffinized with xylene and ethanol and rehydrated with distilled water. Staining of the slides was carried out by immersing the sections in a 0.5% aqueous solution of Toluidine blue for a few minutes, under visual control of the staining intesity. Subsequently, sections were rinsed in distilled water, then dehydrated, cleared in xylene, and coverslipped in Entellan.

For the application of Bismarck brown staining we used the modified protocol of Tomov and Dimitrov [13]. According to it, the sections were deparaffinized with xylene and rehydrated to 70% ethanol. Then they were immersed in a solution of 500 mg Bismarck brown in 80 ml 96% ethanol and 10 ml 1N HCl for 2 hours at room temperature. Following a three-time differentiation in 70% ethanol, the sections were counterstained with Mayer's haematoxylin solution. Thereafter they were dehydrated, cleared in xylene, and coverslipped in Entellan.

Serial sections with both staining techniques were analyzed as the positive mast cells have been calculated for a greater precision in an area of 0.25 mm^2. To compare the results from both staining methods we applied the Student's t-test. Statistical analysis was carried out by SigmaStat® 11.0 software and $p<0.05$ value was accepted as a significant difference.

The specimens were observed and photographed with a Nikon research microscope equipped with a DXM 1200c digital camera.

Results

The CB in rats is bilaterally located at the bifurcation of each common carotid artery, between the external and internal carotid arteries **(Fig. 1A)**. The organ is composed of cell clusters, blood vessels, connective tissue and nerve fibres **(Fig. 1B).** The clusters comprised of two cell types, i.e. neuron-like type I or glomus cells and glial-like type II or sustentacular cells. Postganglionic sympathetic neurons innervating the CB via the ganglioglomerular nerves have their cell bodies in the superior cervical ganglion **(Fig. 1A)**.

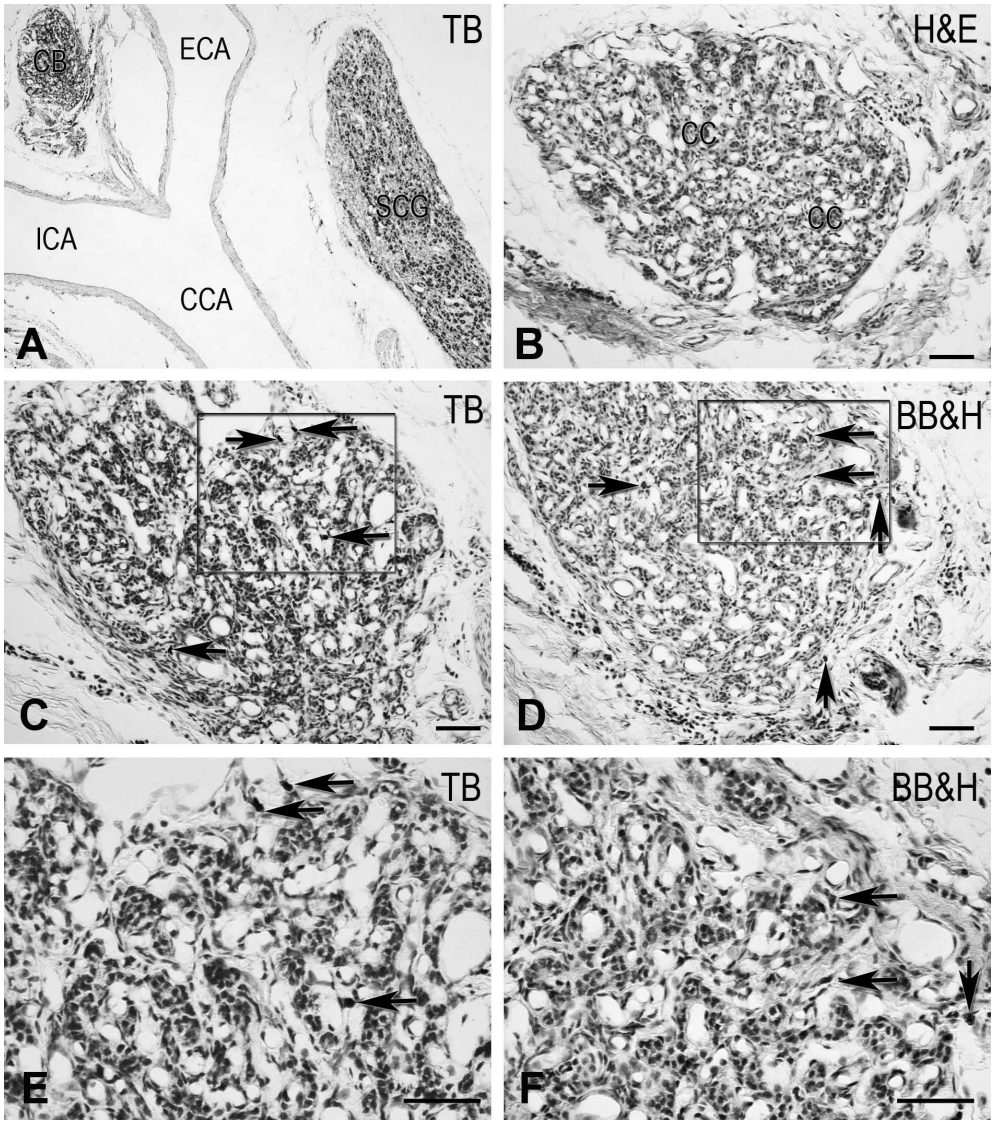

Fig. 1. Localization and distribution of mast cells in the rat carotid body (CB). **(A)** A representative Toluidine blue-stained section of the common carotid artery (CCA) bifurcation area showing the localization of the CB between the external carotid artery (ECA) and the internal carotid artery (ICA) and the superior cervical ganglion (SCG). **(B)** H&E staining illustrates the organization of the rat CB. The glomic tissue is arranged in cell clusters (CC). Low- **(C)** and high- **(E)** power photomicrographs of the Toluidine blue-stained (TB) mast cells (arrows) in the rat CB shown in **A** and **B** displaying the distribution of mast cells. Note that they are predominantly located in the interlobular stroma of the CB and are closely associated with blood vessels. **(F)** Higher magnification of the boxed area in **(D)** demonstrating Bismark brown-stained (BB) mast cells (arrows). Scale bars = 50 μm.

To evaluate the distribution of MCs in the CB of Wistar rats, we applied two classical histological staining techniques for their visualization, i.e. Toluidine blue **(Fig. 1A, C, E)** and Bismarck brown **(Fig. 1D, F).** We observed that the MCs were predominantly located in the interlobular stroma of the CB and were closely associated with blood vessels and no MCs were found within the cell clusters. In particular, a few of the observed MCs were tightly packed to the covering shell of sustentacular cells but were not related to the central core of glomus cells. The average density and distribution of MCs per unit area of 0.25 mm^2 in the rat CB, for both types of staining, was similar: 5.778±0.339 (TB-positive MCs) and 5.929±0.412 (BB-positive MCs) **(Fig. 2).**

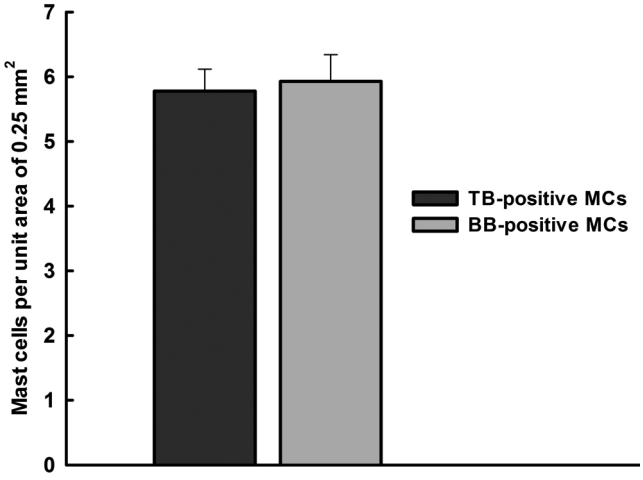

Fig. 2. Vertical bar chart shows the distribution of stained with Toluidine blue and Bismarck Brown mast cells per unit area of 0.25 mm^2. Data are presented as mean±SEM.

Discussion

The results of our study confirm the observations of previous authors [6, 10, 11] that MCs are present in the CB of humans and other mammals. In addition, their number is species-specific and they most frequently occur in bovines [1]. When comparing our data on MC density per unit area in rats (20 – 25 mast cells/mm^2) with the data of other authors [10] on MC density in human CBs (20 – 60 mast cells/mm^2, and mainly in a narrower range of 30 – 60/mm^2) [10], it appears that in the human CB there is a greater MC density and the cells are more abundant than in the rat. Although a difference in their density in the CB of rats and humans exists, it is interesting to point out that distributional patterns remain similar. The MCs are mostly restricted to the interlobular connective tissue where they are often closely related to the small blood vessels. A few MCs are found in a close apposition to the cell islands, but even in that case they are predominantly associated with the sustentacular glial cells. Given that MCs are not directly related to neuron-like glomus cells they are probably not involved in regulating the function of the glomus cells. However, this does not exclude their involvement in the control of small blood vessels and the blood supply within the CB.

The density of MCs remains constant throughout life and is not affected by age and age-related histological appearance of the CB that develops in time. Moreover, certain histopathological conditions involving the CB such as systemic hypertension and chronic obstructive lung disease [8, 12] or coarctation of the aorta [9] do not affect the density and distribution of the MCs.

Conclusion

It can be inferred that the MCs are a constant population in the rat CB and they are most probably related to the functional control of its blood flow via the vasoactive substances they liberate. Further efforts are needed to elucidate their exact chemical nature and physiological mechanism.

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References

1. **Arias-Stella, J., F. Bustos.** Chronic hypoxia and chemodectomas in bovines at high altitudes. - *Arch. Pathol. Lab. Med.,* **100,** 1976, 636-639.
2. **Atanasova, D. Y., M. E. Iliev, N. E. Lazarov.** Morphology of the rat carotid body. - *Biomed. Rev.,* **22,** 2011, 41-55.
3. **Chiocchio, S. R., A. M. Biscardi, J. H Tramezzani.** Catecholamines in the carotid body of the cat. - *Nature, Lond.,* **212,** 1966, 834-835.
4. **Dimitrov, N.** Normal morphology of biologically active point BAP/ST36 rat. - *Acta morphol. et anthropol.,* **19**, 2012, 34-37.
5. **Gonzaez, C., L. A1maraz, A. Obeso, R. Rigual.** Carotid body chemoreceptors: from natural stimuli to sensory discharges. - *Physiol. Rev.,* **74**, 1994, 829-898.
6. **Grimley, P. M., G. G.** Glenner. Histology and ultrastructure of carotid body paragangliomas. Comparison with the normal gland. - *Cancer,* **20,** 1967,1473-1488.
7. **Grönblad, M., P. Liesi, L. Rechardt.** Serotonin-like immunoreactivity in the rat carotid body. - *Brain Res.,* **276,** 1983, 348-350.
8. **Heath, D., P. Smith, R. Jago.** Hyperplasia of the carotid body. - *J. Pathol.,* **138,** 1982, 115-127.
9. **Heath, D., P. Smith, G. Hurst.** The carotid bodies in coarctation of the aorta. - *Br. J. Dis. Chest.,* **80,** 1986, 122-130.
10. **Heath, D., P. Lowe, P. Smith.** Mast cells in the human carotid body. - *J. Clin. Pathol.,* **40,** 1987, 9-12.
11. **Pryse-Davies, J. I., I. M. P. Dawson, G. Westbury.** Some morphologic, histochemical and chemical observations on chemodectomas and the normal carotid body, including a study of the chromaffin reaction and possible ganglion cell elements. - *Cancer,* **17,** 1964, 195-202.
12. **Smith, P., R. Jago, D. Heath.** Anatomical variation and quantitative histology of the normal and enlarged carotid body. - *J. Pathol.,* **137,** 1982, 287-304.
13. **Tomov, N., N. Dimitrov.** Modified Bismarck brown staining for demonstration of soft tissue mast cells. - *Trakia J. Sci.,* **3,** 2017, 195-197.