DOPAMINE AND HISTAMINE: TWO MAJOR TRANSMITTERS IN HYPOXIC CHEMOSENSITIVITY IN THE HUMAN CAROTID BODY

NIKOLAI ELENKOV LAZAROV[1,2], DIMITRINKA YORDANOVA ATANASOVA[2], SEBASTIAN REINDL[3], MANFRED GRATZL[3]

1Department of Anatomy and Histology, Medical University-Sofia, Sofia, Bulgaria
2Institut of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
3Anatomisches Institut, Universität München, München, Germany

Abstract

The carotid body (CB) is the only chemoreceptor sensitive to systemic hypoxia in humans. It has been proposed that several transmitter candidates are released upon hypoxia by the glomus cells of the CB. Evidence to date suggests that dopamine and histamine may serve as transmitters between the chemoreceptor cells and afferent nerve terminals in most animal species. Here we report the expression of markers for the dopamine and histamine metabolism, transport and corresponding receptors in human CBs. In particular, our experiments revealed the existence of dopaminergic and histaminergic traits in the CB chemosensory cells. The catecholamine synthesizing enzyme, tyrosine hydroxylase, and vesicular monoamine transporter 1 were only observed in a subset of dark glomus cells, while histidine decarboxylase, the histamine synthesizing enzyme, and vesicular monoamine transporter 2, which is highly specific for histamine, were detected in virtually all chemosensory cells within the CB glomera. In nerve fibers within and around the glomic lobules we also observed immunoreactivity for a synaptosome-associated protein of 25 kDa, an important component of the neuroendocrine exocytotic apparatus. In addition, the majority of chemoreceptor cells in the human CB were richly endowed with dopamine D2 receptors and histamine receptors 1 and 3. The present data show that human glomus cells from adult individuals express all the components for the biosynthesis, storage and release of catecholamines and histamine, as well as their receptors. In conclusion, it can be inferred that dopamine and histamine are the primary transmitters in hypoxic chemosensitivity in humans.

Keywords: carotid body, chemoreception, dopamine, histamine, human.

Introduction

The carotid body (CB) is the major arterial oxygen sensor in humans that plays essential roles in the blood gas and pH homeostatic control, initiating an appropriate respiratory and cardiovascular response to hypoxia, hypercapnia and acidosis. It is a small paired organ strategically positioned at the bifurcation of each common carotid artery. The CB consists of two main cell types: neural crest-derived type I (also called glomus) chemosensory cells, which contain secretory granules, and type II (or sustentacular) cells, which are supporting glial-like cells [1] and recently proposed to be CB stem cells [2, 3]. These two cell types are juxtaposed and together make up small clusters called

glomeruli or glomoids (Fig. 1). On the other hand, glomus cells are synaptically connected to the nerve endings of petrosal ganglion neurons, thus ensuring the transmission of the chemosensory information from peripheral arterial chemoreceptors to the central nervous system. The efferent limb of the chemoreceptor reflex arc is formed by solitary axons projecting to the respiratory control centers, distributed in a ponto-medullary respiratory network. They control the coordinated contractions of the abdominal, thoracic and laryngeal respiratory muscles.

It has been proposed that several transmitter candidates are released upon hypoxia by the glomus cells of the CB in different animal species. In their turn, the neurotransmitters also contribute to the modulation of glomus cell function via autoreceptors. However, there are species differences regarding the expression of various transmitters and their corresponding receptors in the CB which may result in variations of chemosensory signaling. On the other hand, since the CB is not fully developed at birth, plasticity-induced neurochemical changes may occur later in life [4]. As a result, the change in neurotransmitter or receptor profiles in the CB during maturation may cause altered CB responses to hypoxia [5]. Moreover, as human infants seem particularly vulnerable to hypoxic and hypercapnic episodes during sleep, cellular alterations in peripheral chemoreceptors resulting in altered chemosensitivity may be one of the factors contributing to a higher incidence of sudden infant death syndrome in premature newborns [4].

Biogenic amines are considered to be primary messengers at the junctions between glomus cells and nerve terminals [1]. In particular, dopamine is considered an important inhibitory modulator of chemoreceptor activity in most mammalian species [1, 6-10]. Previous research has also shown that in man it plays a significant role in ventilatory adaptation to hypoxia [11]. In addition to one report about the localization of tyrosine hydroxylase (TH), the rate-limiting enzyme for catecholamine synthesis, in glomus cells and nerve fibers in the human CB [12], the presence of dopamine, its traits and receptors in humans of different ages have lately been reported [13]. Recently, histamine has also been implicated in hypoxic chemosensitivity in rats [14] and cats [15]. Its actions are mediated by at least four G-protein-coupled receptor subtypes encoded by different genes referred to as H1-H4. In this study we have investigated the dopaminergic and histaminergic traits in the human mature CB, with a particular focus on the role of dopamine and histamine in hypoxic chemoreception.

Materials and Methods

The experiments were carried out on human CB samples obtained at routine autopsies from six adult patients of both sexes. Their age ranged from 20 to 52 years and the time elapsing before tissue fixation did not exceeded 16 h. The carotid bifurcations were excised, both CBs were immediately dissected out, specimens were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 and tissue blocks were embedded in paraffin, cut at 5 μm thick sections and subsequently processed for ABC (avidin-biotin-horseradish peroxidase complex) immunohistochemistry. Briefly, following antigen retrieval in 10 mM citrate buffer, pH 6.0 in a microwave oven, the sections were preincubated in 5% normal goat serum to avoid nonspecific staining, and treated with ABC blocking kit (Vector Laboratories Inc., Burlingame, CA, USA) to block unspecific biotin. Afterwards, they were incubated in a humid chamber overnight at 4°C with primary antibodies against histidine decarboxylase (HDC; Progen Biotechnik GmbH, Heidelberg, Germany), histamine (HIS; Sigma, St. Louis, MO), human histamine 1 receptor (H1R; Acris Antibodies GmbH, Hiddenhausen, Germany), histamine 2 receptor (H2R; Alpha Diagnostics, San Antonio, TX), histamine 3 receptor (H3R), histamine 4 receptor (H4R; both from Abcam Ltd., Cambridge, UK), vesicular monoamine transporter 1 (VMAT1) and vesicular monoamine transporter 2 (VMAT2; both from Phoenix Pharmaceutical Inc., Belmont, CA), rabbit polyclonal antiserum to dopamine D2 receptor (D2R; BIOTREND Chemikalien GmbH, Köln, Germany), mouse monoclonal antibodies to TH (LOXO GmbH, Dossenheim, Germany), dopamine (Abcam) and synaptosome-associated protein of 25 kDa (SNAP25; SMI, Lutherville, MR). After rinsing in phosphate buffered saline, the sections were reacted with the respective secondary antibody, biotinylated goat anti-rabbit IgG or goat anti-mouse IgG (both from Dianova, Hamburg, Germany) and then the ABC-complex (Vectastain Elite Kit; Vector) was applied. After color development the sections were coverslipped with Entellan through alcohols and xylene. Finally, the specimens were examined and

photographed with a Zeiss research microscope. The specificities of antibodies used and control staining applied in this study have been described in detail previously [13].

Results

Immunoreactive for dopamine cells were distributed throughout the human mature CB and characteristically appeared as cell clusters. In particular, a subset of dark glomus cells was immunoreactive for TH, the catecholamine synthesizing enzyme (Fig. 2), as well as for the dopamine molecule. Likewise, relatively few type I cells, some of them TH-containing, were also immunopositive for the other dopaminergic traits, i.e. VMAT1 (Fig. 3), a specific transporter for catecholamines, and SNAP25 (Fig. 4), an important component of the neuroendocrine exocytotic apparatus, that was localized on nerve fibers within and around the glomic lobules in the CB. Conversely, the immunohistochemical experiments demonstrated immunoreactivity for D2-dopamine receptor in a much greater number of glomus cells in comparison with TH-containing cells in the fully developed CB (not shown).

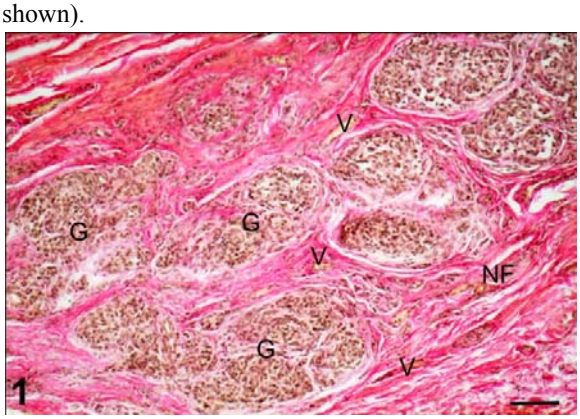

Fig. 1 Routine van Gieson staining illustrating the normal morphology of the adult human CB. Note the compact glomic lobules (G) surrounded by septa of connective tissue. A large number of blood vessels (V) and nerve fibers (NF) can also be seen. Scale bar = 100 µm.

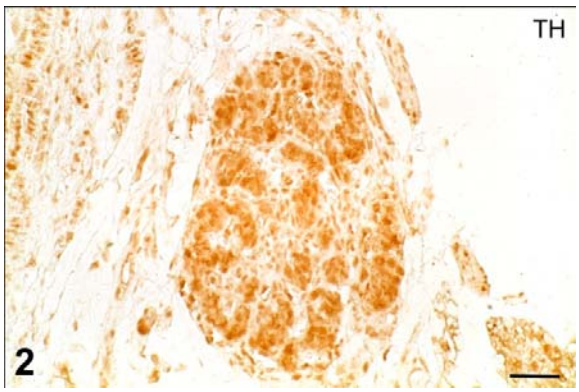

Fig. 2 Immunohistochemical staining for tyrosine hydroxylase (TH) in the mature human CB. Only a few glomus cells are immunoreactive for the catecholamine synthesizing enzyme. Scale bar = 100 µm.

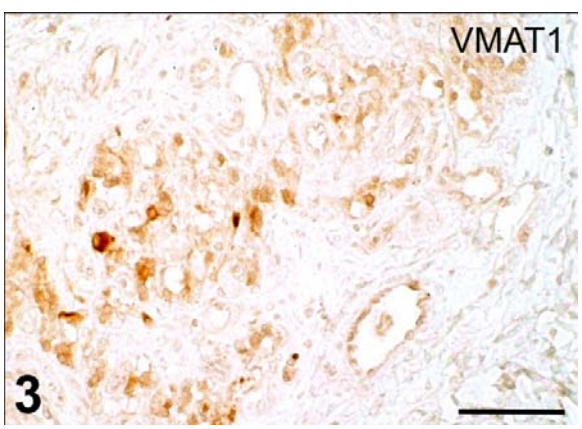

Fig. 3 VMAT1 immunoreactivity in a subset of type I cells in adult human CB. Scale bar = 100 µm.

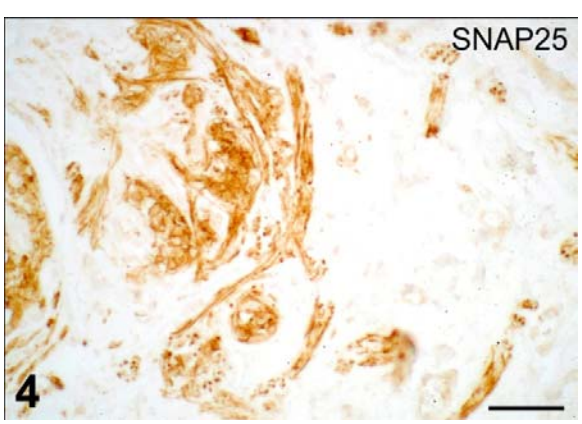

Fig. 4 SNAP25-immunopositive glomus cells and nerve fibers within and around the glomic lobules. Scale bar = 50 µm.

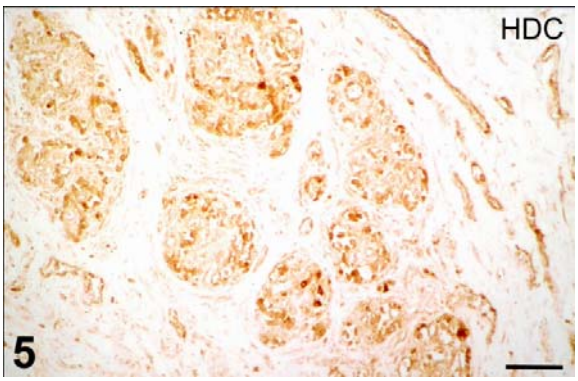

Fig. 5 Expression of histidine decarboxylase (HDC) in the adult human CB. A vast majority of glomus cells in the glomeruli exhibit strong immunoreactivity for the histamine synthesizing enzyme. Scale bar = 100 μm.

Using antibodies directed against histamine itself and against HDC, the enzyme necessary for histamine synthesis, we identified a large number of histaminergic cells, typically aggregated in cell clusters (Fig. 5). In addition, almost all glomus cells were immunoreactive for VMAT2, which is highly specific for histamine (Fig. 6). Our results also showed that relatively more type I cells within the glomera of adult humans expressed H1 and H3, but not H2 and H4, histamine receptor proteins. No immunoreaction to any of the tested antigens was detected in the tissues when normal serum instead of a primary antiserum was used (not shown).

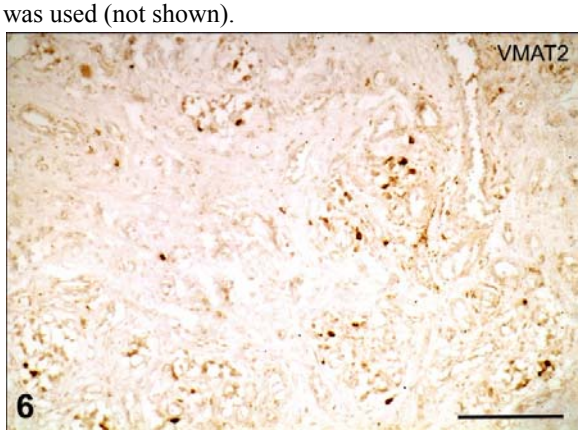

Fig. 6 Most of the histamine-containing cells are intensely VMAT2 immunostained. Scale bar = 100 μm.

Discussion

The results of this study provide immunohistochemical evidence that glomus cell, regardless of their postmortem structural changes [16, 17], express all the biochemical components for the biosynthesis, storage and release of dopamine and histamine upon hypoxia as well as show the existence of certain specific receptors at the presynaptic and/or postsynaptic levels in the human CB.

Investigations of CBs in many different species, during various stages of development, have led to the conclusion that dopamine is a likely primary transmitter in the CB, because it meets most of the necessary criteria for such a role including biosynthesis and storage of dopamine, as well as Ca2+-dependent release triggered by hypoxia. Our present findings on the expression of dopamine, its components of exocytotic apparatus and dopamine receptors allow for more definitive characterization of dopaminergic profiles of glomus cells involved in hypoxic chemosensitivity. Moreover, expression of inhibitory, hyperpolarizing presynaptic D2 autoreceptors on the glomus cells confirm that dopamine may serve as an inhibitory modulator of the transmitter(s) responsible for the afferent sensory activity upon hypoxia (see [6, 8, 10], and references therein). However, though dopamine has already been found to be the major amine at birth [11], we were not able to prove the postnatal developmental enhancement of dopaminergic traits and changes in oxygen responsiveness, reported by Gauda and Lawson [5]. Thus, dopamine does not seem to be directly involved in the maturational processes of CB oxygen sensitivity in man.

On the other hand, several lines of evidence suggest that histamine could be more essential than dopamine in hypoxic transmission during postnatal development in humans. First, radioenzymatic and immunohistochemical evidence points out that the storage of histamine in the glomus cells exceeds that of dopamine more than 10-fold [18]. Secondly, here we show that histaminergic traits tend to be expressed in virtually all glomus cells of adult humans. Thirdly, our data also demonstrate that a substantially greater number of chemoreceptor glomus cells are richly endowed with histamine H1- and H3 receptors. It is likely that the signal transmission of the human glomus cells may be differentially modulated at the presynaptic level by histamine through excitatory H1 and inhibitory H3 autoreceptors.

In conclusion, it can be inferred that histamine and dopamine are important transmitters in hypoxic chemosensitivity in man acting via certain

corresponding receptors (H1, H3 and D2, respectively). Furthermore, the changes in their levels may play important roles in the maturation of the physiological function of the carotid chemoreceptors in response to hypoxia in humans.

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Figure captions