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Neurotransmission in the Human Carotid Body: Focus on the Role of Dopamine and Histamine in Hypoxic Chemoreception

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The carotid body (CB) is the only chemoreceptor sensitive to systemic hypoxia in humans. Its physiological action is regulated by multiple neurotransmitters, including several biogenic amines. Evidence to date shows an involvement of dopamine as an inhabitory modulator of the chemoreception in man. Histamine, released from glomus cells, has recently been considered a putative transmitter in hypoxic chemosensitivity in rats. In the present study, we investigated the expression of markers for histamine metabolism, transport and corresponding receptors in the human CB and revealed an expression of histidine decarboxylase, synaptosome-associated protein of 25 kDa, vesicular monoamine transporter 2, and histamine receptors 1 and 3 in virtually all chemosensory cells within the glomera. By contrast, dopaminergic traits (tyrosine hydroxylase, vesicular monoamine transporter 1 and D2 receptors) were only detected in a subset of glomus cells. Our data show that histamine, along with dopamine, plays an important role in the chemosensory function in humans.

Key words: carotid body, dopamine, histamine, hypoxia, human.

Introduction

The carotid body (CB) is a major arterial oxygen sensor 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 [5] and recently pro-

posed to be CB stem cells [13]. These two cell types are juxtaposed and together make up small clusters called glomeruli or glomoids. 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 differences in species regarding the expression of various transmitters and their corresponding receptors in the CB which may result in variations of chemosensory signalling. 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 [3]. Moreover, as human infants seem particularly vulnerable to hypoxic and hypercapneic 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 in the junctions between glomus cells and nerve terminals [5]. In particular, dopamine is considered an important inhabitory modulator of chemoreceptor activity in most mammalian species; previous research has shown that in man it plays a significant role in ventilatory adaptation to hypoxia [2, 7, 8, 12]. Nonetheless, with the exception of 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 [10] the full biochemical machinery for dopamine storage and release, as well as specific dopamine receptors, have not been localized there so far. Recently, histamine has also been implicated in hypoxic chemosensitivity in rats [9, 11]. 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 chemosensory traits in the human CB of different ages 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 nine patients of both sexes. Their age ranged from 4 months to 76 years and the time elapsing before tissue fixation did not exceeded 48 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 cham-

ber 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 [9, 11].

Results

Immunoreactive for dopamine cells were distributed throughout the human CB of different ages and characteristically appeared as cell clusters. In particular, a subset of dark glomus cells in both immature and mature CB was immunoreactive for TH, the catecholamine synthesizing enzyme, as well as for the dopamine molecule (Fig. 1A, B). Likewise, relatively few type I cells, some of them TH-containing, were also immunopositive for the other dopaminergic traits, i.e. VMAT1, transporting catecholamines and SNAP25, an important component of the neuroendocrine exocytosis apparatus, that was localized on nerve fibers within and around the glomic lobules in the CB (Fig. 1C-F). 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 both infantile and fully developed CBs (Fig. 1G, H).

In general, the distribution of histaminergic traits and the intensity of immunostained cells in the juvenile CB was essentially the same as that of the adult CB. Using antibodies directed against histamine itself and against HDC, the enzyme necessary for histamine synthesis, we identified a large number of histaminergic cells in both the immature and mature CB, typically aggregated in cell clusters (Fig. 2A, B). In addition, almost all glomus cells were immunoreactive for VMAT2, which is highly specific for histamine (Fig. 2C, D). Our results also showed that relatively more type I cells within the glomera of different ages expressed H1 (Fig. 2E, F) and H3 (Fig. 2G, H), 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).

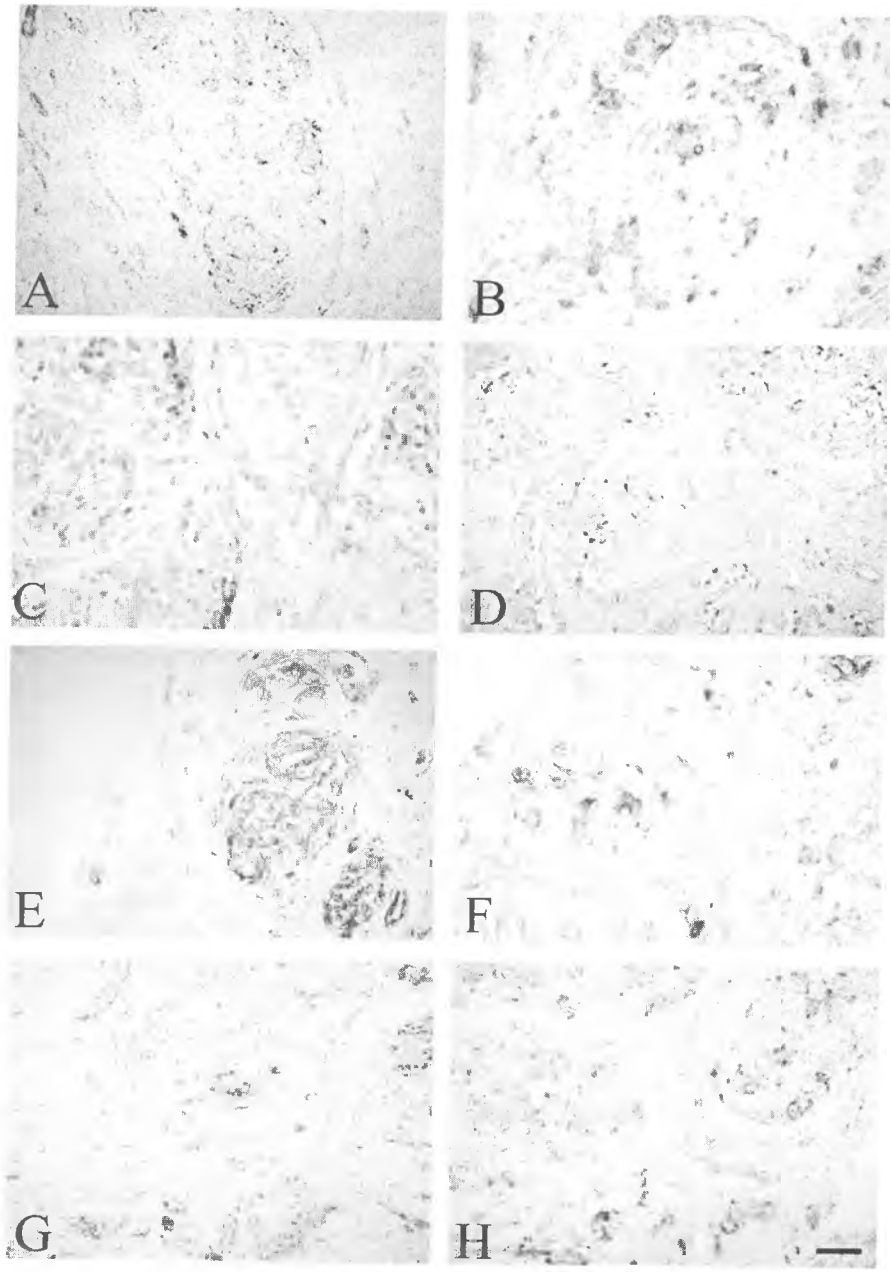

Fig. 1. Expression of dopamine and dopaminergic traits in the infantile (**A, C, E, G**) and adult (**B, D, F, H**) human CB. Immunohistochemical staining for dopamine in the immature (**A**) and mature (**B**) CB. Note that only a few glomus cells are immunoreactive with no age differences in their number and intensity of staining. (**C**) and (**D**) show the VMAT1 immunoreactivity in a subset of type I cells in childish and adult CB, respectively. (**E, F**) SNAP25-immunopositive glomus cells and nerve fibers within and around the glomic lobules are also observed. Relatively more numerous glomus cells contain D2 receptor protein (**G, H**). Scale bars = 100 μm

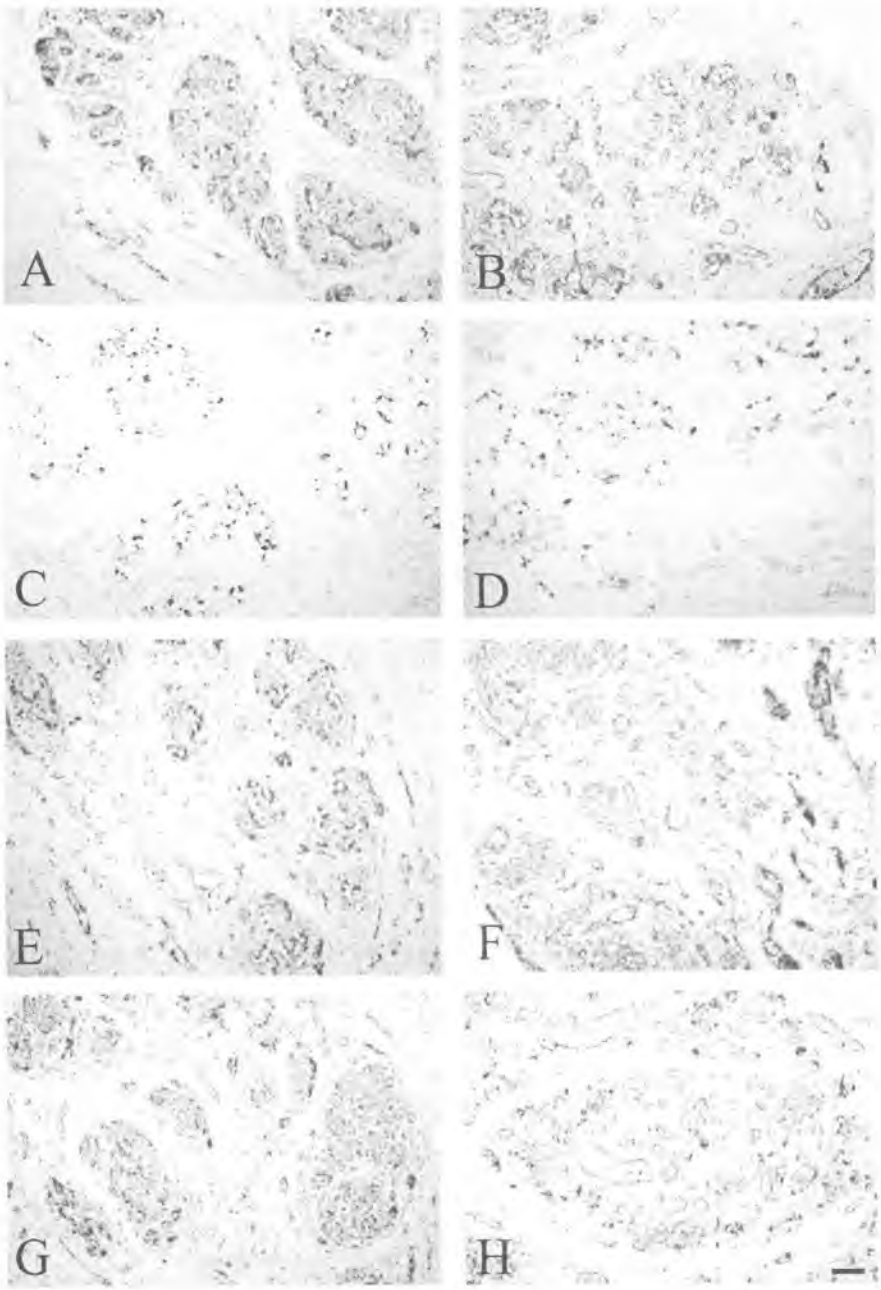

Fig. 2. Expression of histamine and histaminergic traits in the infantile (**A, C, E, G**) and adult (**B, D, F, H**) human CB. Microphotographs at low magnifications showing the presence of histamine in the immature (**A**) and mature (**B**) CB. A vast majority of glomus cells in the glomeruli exhibit strong immunoreactivity for histamine molecule with similar distributional patterns. (**C, D**) Also, most of the histamine-containing cells are intensely VMAT2 immunostained. A large number of glomus cells are abundantly endowed with H1 (**E, F**) and H3 (**G, H**) receptors. Scale bars = 100 μm

Discussion

The results of our study provide the first immunohistochemical evidence that glomus cell, regardless of their postmortem structural changes [6, 14], express all the biochemical components for biosynthesis, storage and release of dopamine and histamine upon hypoxia as well as the existence of certain specific receptors at the presynaptic and/or postsynaptic levels in the human CB. Nonetheless, the distributional patterns of dopaminergic and histaminergic traits do not differ with age, indicating that aminergic profiles of human CB glomus cells are not age-dependent.

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 Ca^{2+}-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 inhabitory, hyperpolarizing presynaptic D2 autoreceptors on the glomus cells confirm that dopamine may serve as an inhibitory modulator of the transmitter(s) responsible for afferent sensory activity upon hypoxia (see [2, 8], and references therein). However, though dopamine has already been found to be the major amine at birth [1] we were not able to prove the postnatal developmental enhancement of dopaminergic traits and changes in oxygen responsiveness, reported by Gauda and Lawson [3]. 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 can be more essential than dopamine in hypoxic transmission during postnatal development in humans. Firstly, radioenzymatic and immunohistochemical evidence points out that storage of histamine in the glomus cells exceeds that of dopamine more than 10-fold [9]. Secondly, here we show that histaminergic traits tend to be expressed in virtually all glomus cells of young and adult humans. Thirdly, our data also demonstrates that a substantially greater number of chemoreceptor glomus cells are richly endowed with histamine H1- and H3 receptors. It is likely that 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 carotid chemoreceptors in response to hypoxia in humans.

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