Selective Expression of Galanin in Neuronal-Like Cells of the Human Carotid Body

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Abstract

The carotid body is a neural-crest-derived organ devoted to respiratory homeostasis through sensing changes in blood oxygen levels. The sensory units are the glomeruli composed of clusters of neuronal-like (type I) cells surrounded by glial-like (type II) cells. During chronic hypoxia, the carotid body shows growth, with increasing neuronal-like cell numbers. We are interested in the signals involved in the mechanisms that underlie such response, because they are not well understood and described. Considering that, in literature, galanin is involved in neurotrophic or neuroprotective role in cell proliferation and is expressed in animal carotid body, we investigated its expression in human. Here, we have shown the expression and localisation of galanin in the human carotid body.

Keywords

Galanin • Carotid body • Hypoxia • Oxygen sensing

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36.1 Introduction

The carotid body (CB) is a chemosensitive structure for acute oxygen-sensing and oxygen homeostasis that is key to life. It is a paired organ that is positioned at the bifurcation of the carotid artery, and during development it originates from the neural crest. The CB is innervated by afferent sensory nerve fibres that are connected to the glossopharyngeal nerve, which activate the brainstem respiratory centre to produce hyperventilation during hypoxaemia. The CB chemosensing core constitutes an intricate network of blood vessels that originate from a branch from the external carotid artery, and which spread into the parenchyma of the CB, where they make contact with the glomeruli. The glomeruli are the chemosensory units that are formed by a cluster of cells: the neuronal-like, or type I, cells, that are surrounded by the processes of non-excitable glial-like, sustentacular, or type II, cells. The neuronal-like cells are in tight contact with a minute branch off the capillaries, and they show some similarities to sympathetic neurons. They are electrically excitable, express oxygen-sensitive K^+ channels and an inward Ca^{2+} current, which contributes to the membrane depolarisation that results in neurotransmitter secretion through dense-core vesicles (Lopez-Barneo et al. 2001; Peers and Buckler 1995; Weir et al. 2005).

Under chronic hypoxia, the CB can enlarge to several-fold its normal size, as also seen in patients with cardiopulmonary disease (Wang and Bisgard 2002; McGregor et al. 1984; Heath et al. 1982). This occurs through the production of new neuronal-like cells by activation of a resident population of neural-crest-derived progenitor cells. Furthermore, on returning to normoxia conditions, the original size of the CB is restored, with about half of the CB glomus cell mass replaced by these newly formed cells This thus indicates a striking regenerative power that is unusual for adult neural tissue.

These newly formed cells have the same neurochemical complex and electrophysiological properties as the mature neuronal-like cells. Consequently, the CB has been considered to be a neurogenic centre with a recognisable physiological function in adult life, because it contains such self-renewing and multipotent stem cells, which putatively represent the glial cells (Pardal et al. 2007).

In a similar chemosensory organ to the CB, the olfactory system, the generation of new olfactory neurons in adult mouse brain is driven by galanin (Cordeo-Llana et al. 2014). Furthermore, galanin has been described in the animal carotid body (Kameda 1989; Heym and Kummer 1989; Ichikawa and Helke 1993; Finley et al. 1995), and the galanin receptors GalR1 and GalR2 have been shown to be expressed in rat CB type I cells (Porzionato et al. 2010). We have therefore investigated whether galanin is expressed in the human CB.

36.2 Methods

Human carotid bodies (n = 12) were collected from 12-h to 72-h post-mortem, for subjects (mean age, 50 years; age range, 26.5–75.5 years) who had not shown chronic pulmonary or cardiovascular disease. The exclusion criteria were for signs at autopsy examination that the subject had undergone cardiac hypertrophy or previous myocardial infarction, and for subjects who showed any tissue degeneration or alteration detected using standard histological staining. Furthermore, the possible influence of the death-to-autopsy interval was examined statistically (for detailed method, see Porzionato et al. 2005). The study was approved by the local Ethics Committee, and was performed according to Italian laws on human autopsy tissue (Porzionato et al. 2005).

The specimens were fixed in neutral 10 % formalin, embedded in paraffin wax, sectioned (5 μm), and examined following Mallory trichrome histological staining (Bio Optica, Milan, Italy) and Azan blue staining (Bio Optica, Milan, Italy). The H-11 mouse monoclonal anti-galanin antibody (sc166431; Santa Cruz Biotechnology, CA, USA), the H1α 67 anti-hypoxia inducible factor (HIF) antibody (sc-53546; Santa Cruz Biotechnology), and developing kits (HRP Polymer/DAB Plus Chromogen, Lab Vision UltraVision LP Detection System; Thermo Scientific, CA, USA) were used for the immunohistochemistry. For light microscopy and data acquisition, a Leica DM 4000

microscope was used, which was equipped with the Leica DFC 320 digital acquisition system (Leica Cambridge Ltd., Cambridge, UK). The QWin Plus 3.5 software (Leica Cambridge Ltd.) was used to digitise the images and to compute the areas positive for the antibodies. Commercial software (i.e., SPSS, Origin) were used for the data and statistical analyses, using one-way ANOVA (α set to 0.001, unless otherwise specified).

36.3 Results

The anatomical organisation of the human CB was investigated using Azan blue staining (Fig. 36.1a, b). The anatomical structure highlighted in the histological staining revealed the CBs were preserved.

Mallory trichrome staining (Fig. 36.2a, b) coupled with immunostaining with an anti-HIF antibody (Fig. 36.3a, b) of the human CB sections

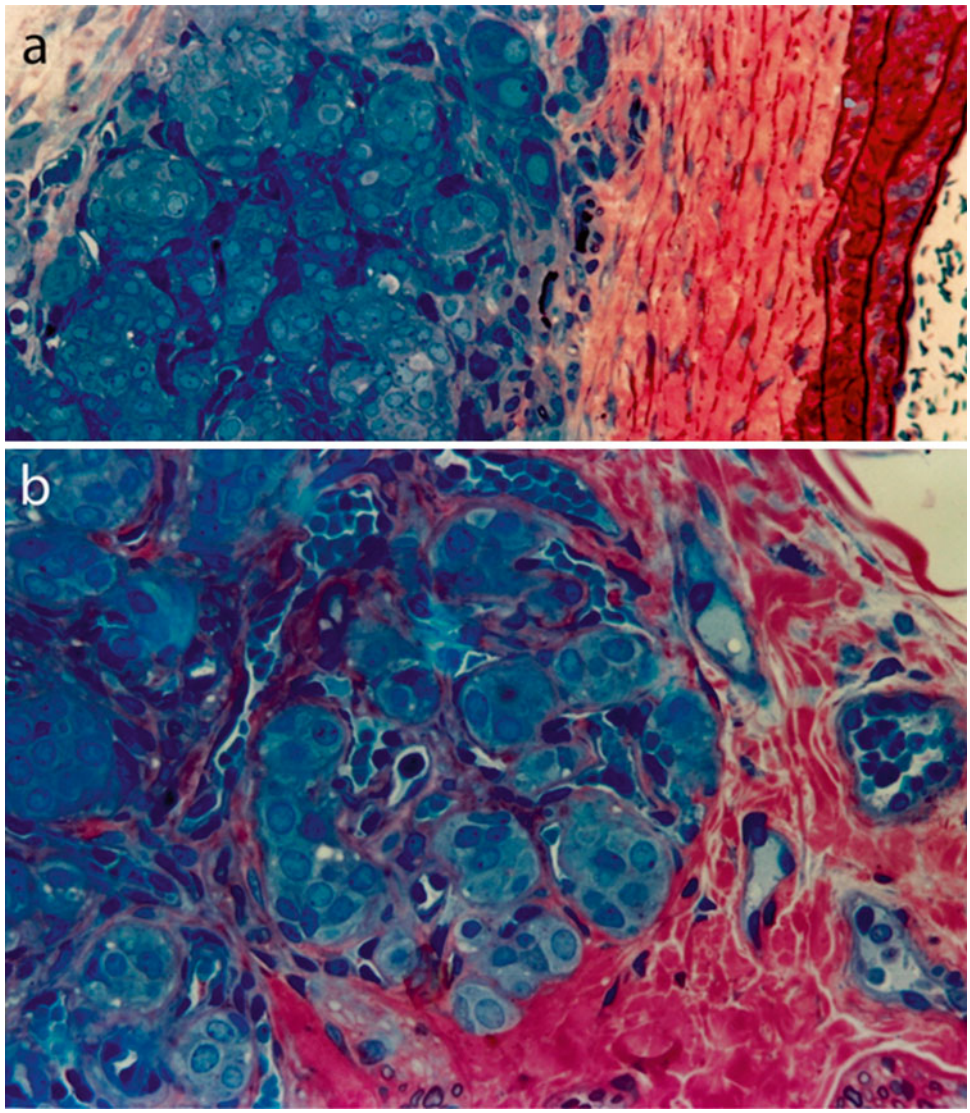

Fig. 36.1 Representative Azan blue staining of the human carotid body (CB) (**a**) histological sections of the human carotid bifurcation shows anatomical organization of CB, (**b**) higher magnification of the glomeruli clusters that form the functional chemosensing unit of the CB

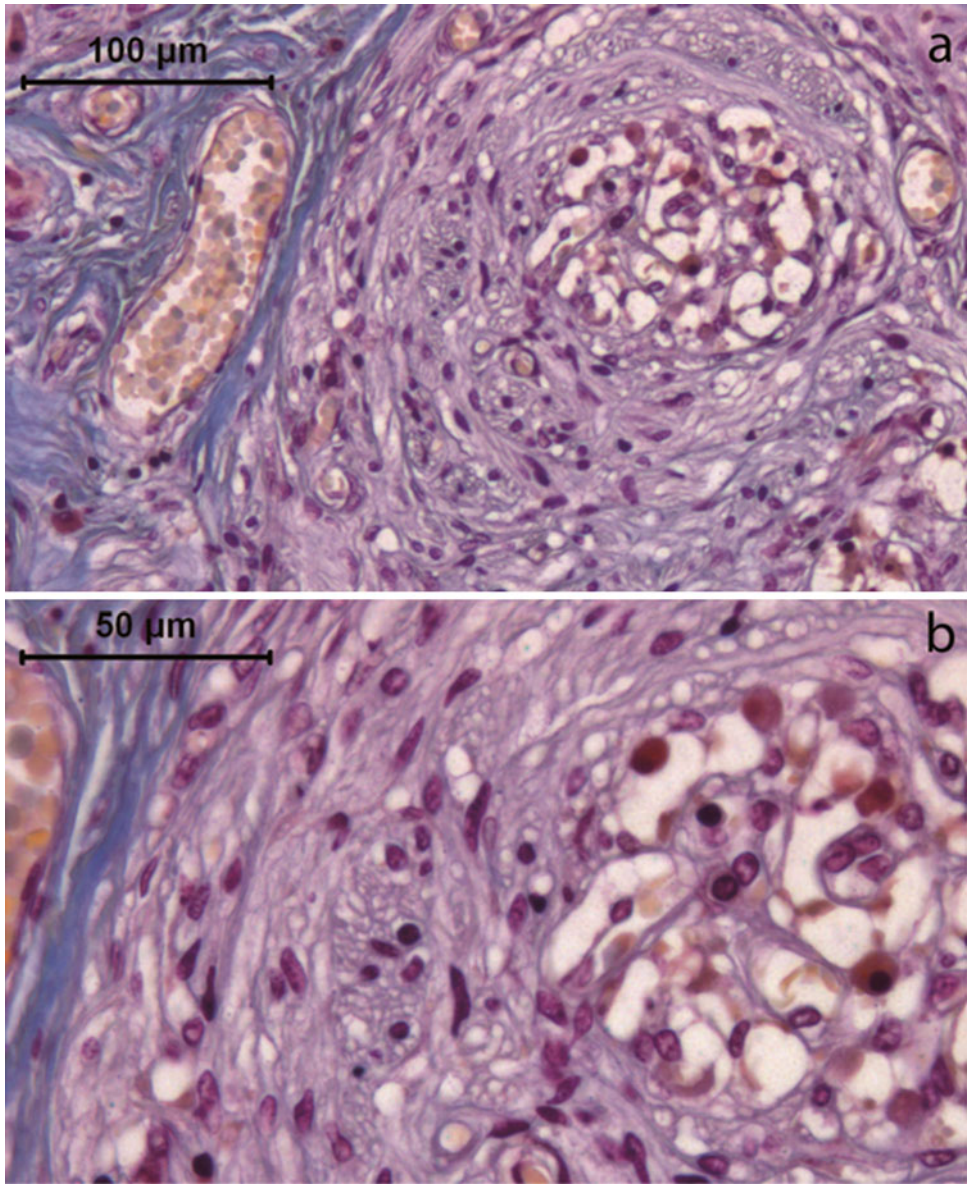

Fig. 36.2 Mallory trichrome staining of human CB sections (**a**) the glomerulus organisation and the blood vessel network are highlighted, (**b**) higher magnification

highlight the organisation of the glomeruli and the blood vessel network. This staining shows a detailed tissue sensorial structure of the CB. The HIF immunostaining, labels specifically the type I cells, and illustrates the functional organization of CB.

Histological sections of the human CB immunostained with an anti-galanin antibody reveal the galanin expression in type I cells, which are anatomically present in the sensorial structure of the CB (Fig. 36.4a, b).

Figure 36.5 shows high magnification of the sensorial unit of the CB highlighted using the bass-relief technique, showing the neuronal-like cells, glial cells, blood vessels and afferent sensory nerve fibres. The inset to Fig. 36.5 shows immunostaining of the neuronal-like cells.

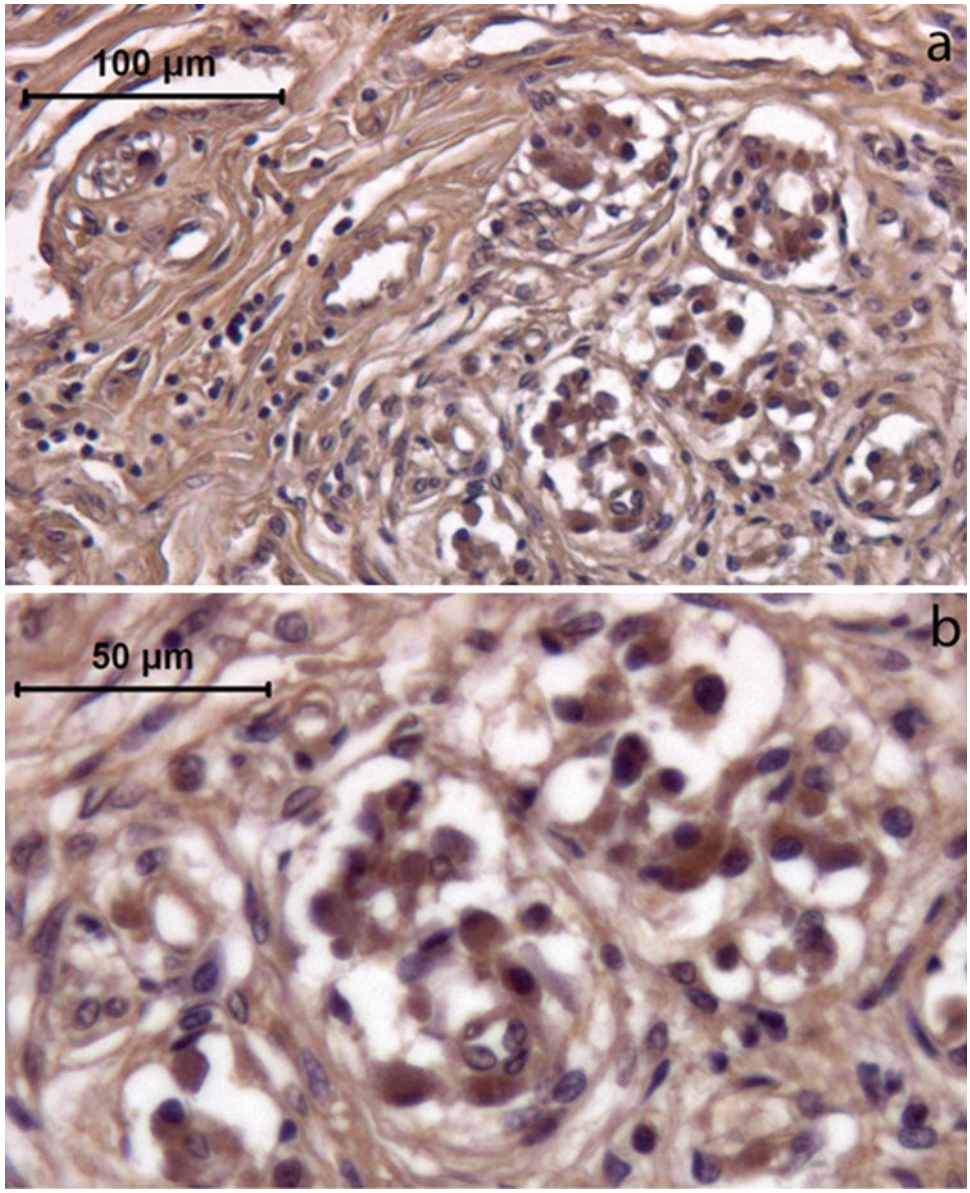

Fig. 36.3 Immunostaining of human CB sections with an anti-HIF antibody (**a**), HIF is selectively expressed by neuronal-like cells (**b**)

36.4 Discussion

We have found here the expression of the neuropeptide galanin in human CB. Galanin was first isolated from porcine intestine (Tatemoto et al. 1983) and was subsequently found in several species and tissues (Ch'ng, et al. 1985; Melander et al. 1986). Galanin expression has been shown throughout both the central and peripheral nervous systems, including the CB of rat, monkey, guinea pig and chicken (Kameda 1989; Heym and Kummer 1989; Ichikawa and Helke 1993; Finley et al. 1995).

The galanin peptide in animals consists of 29 amino acids, while it has 30 amino acids in

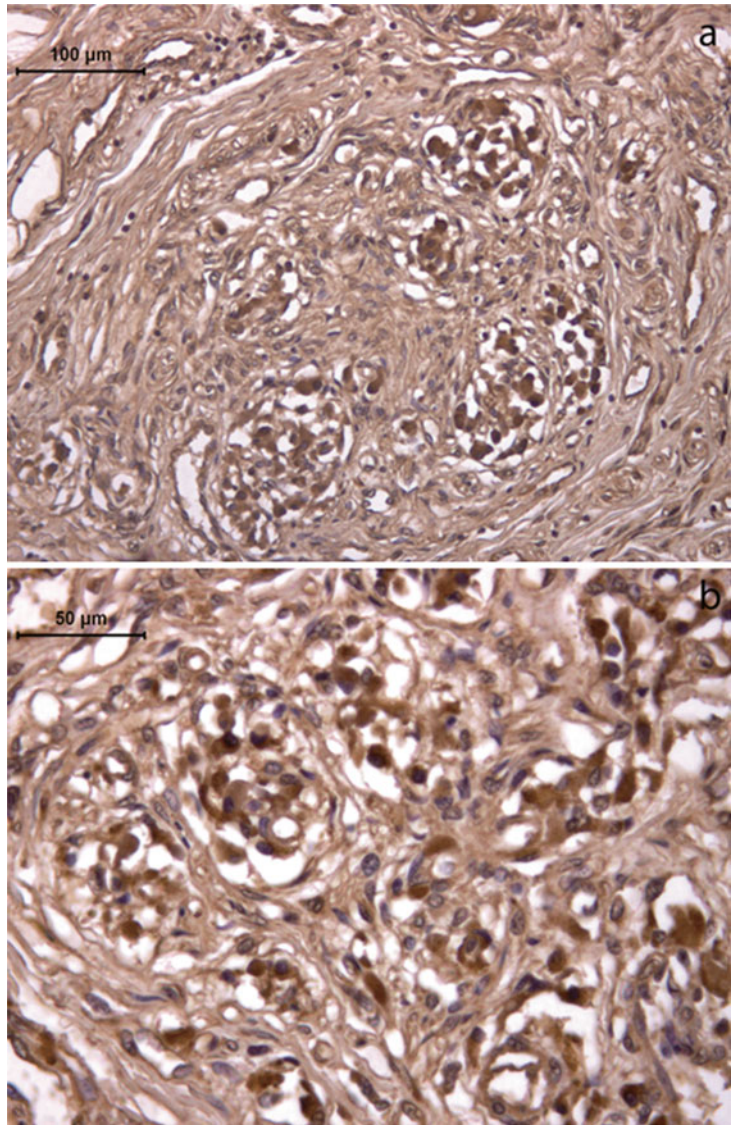

Fig. 36.4 Immunostaining of human CB sections with an anti-galanin antibody (**a**), higher magnification of the galanin expression (**b**)

human. Galanin is a highly inducible neuropeptide that has been conserved across species, and it has been shown to be distinctly up-regulated within the nervous system after pathological disturbance, where its N-terminal region has been shown to be crucial for its biological activity (Branchek et al. 2000). Galanin belongs to the galanin-related peptide family of neuropeptides, which is different from the other neuropeptide families (Ohtaki et al. 1999). Its biological effects are mediated through three different G-protein coupled receptors, termed GalR1, GalR2 and GalR3. Only the first two of these galanin receptors, GalR1 and GalR2, have been shown to be expressed in rat CB (Branchek et al. 2000; Florén et al. 2000; Porzionato et al. 2010).

GalR1 has been shown to be coupled to Gi/o, which acts through inhibition of adenylyl cyclase. However, GalR2 acts through another signalling pathway: via Gq/11 and the activation

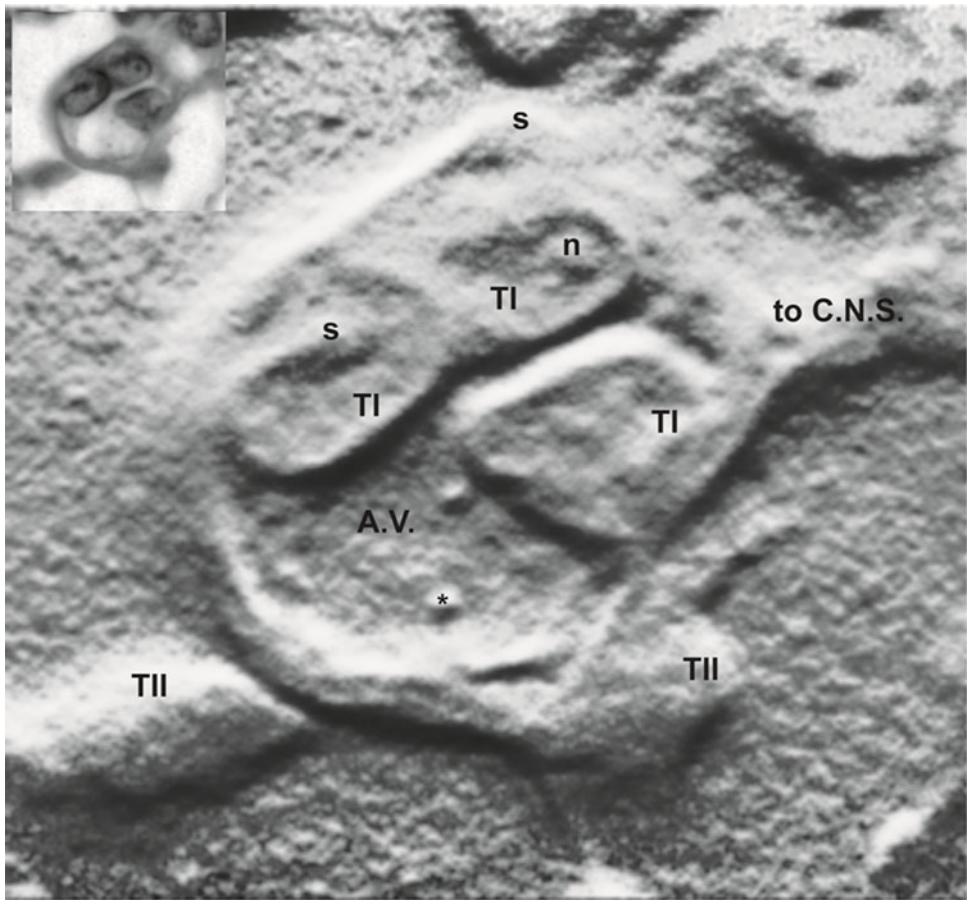

Fig. 36.5 Bass-relief technique to highlight the sensorial unit of the CB (**a**), which is composed of the neuronal-like cells (type I; TI), glial-like cells (type II; TII), artery vessels (*A.V.*), synapses (*s*), and afferent sensory nerve fibres (CNS). *n* nucleus, * erythrocyte. (**b**) *Inset*: Anti-galanin antibody immunostaining of a neuronal-like cell

of phospholipase C and protein kinase C (Wang et al. 1998; Wittau et al. 2000). As such, galanin can participate in the modulation of several ascending neurotransmitter systems, including cholinergic, noradrenergic, and serotonergic pathways (Tatemoto et al. 1983; Crawley et al. 2002).

Galanin acts as a neurotrophic/neuroprotective factor for several neuronal populations, and mainly for sensory neurons, and it is involved in the plasticity of the nervous system (Xu et al. 1996; Wiesenfeld et al. 1992; Hokfelt et al. 1987, 1994; Holmes et al. 2000). Moreover, in vitro galanin administration resulted in up-regulation of genes involved in the pro-survival/ pro-neuronal signalling pathways, and increased the number of neurons upon differentiation from olfactory sensory neuron progenitors (Cordeo-Llana et al. 2014).

Treatment with galanin and the specific agonist Gal2-11 in wild-type and GAL knock-out neural stem cells under differentiation conditions significantly promotes neuritogenesis, which is inhibited by the galanin antagonist M35 (Ma et al. 2008). Thus, galanin regulates differentiating neural stem cells, and in this way it can participate in the development and plasticity of the nervous system.

The CB can undergo size adaptation that leads to an increase under chronic hypoxia condition,

due to the production of new neuronal-like cells. Conversely, when returned to normoxia conditions, there is a corresponding decrease in the size of the CB, whereby the sensory cells have been renewed by the activation of a resident population of neural-crest-derived progenitors (Wang and Bisgard 2002; McGregor et al. 1984; Heath et al. 1982; Pardal et al. 2007). These adaptive phenomena have indicated the presence of self-renewing and multipotent stem cells in the CB (Pardal et al. 2007). The sustentacular cells are glial-like cells, and these express astrocyte markers and have a supportive role (Pallot 1987). When exposed to prolonged hypoxia, it has been proposed that type II cells themselves differentiate, or there is the production of stem-cell precursors of the neuronal-like cells (Pardal et al. 2007). This mechanism is crucial for the physiological role of the CB, because during adult life, this maintains a population of cells that can differentiate into neuronal-like cells.

Following the recent report that galanin is involved in neurodifferentiation of chemosensory neurons (Cordeo-Llana et al. 2014), we here investigated its potential expression in the glomeruli of the human CB, through identification of the cell type(s) that express it, and by examining the cell morphology to determine their position inside the glomeruli. There was no immunostaining for the glial like cells.

In conclusion, we have shown here the expression of galanin in human CB, with its selective expression in the neuronal-like or type I cells.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

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