Original Article

NEUROTRANSMITTERS AND THEIR RECEPTORS IN THE RAT SUPERIOR CERVICAL GANGLION

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Summary

The superior cervical ganglion (SCG), the sympathetic organ innervating the carotid body (CB), lies at the bifurcation of the common carotid artery, near the CB. The ganglion is composed of functional subsets of neurons showing different sympathetic phenotypes. The expression of putative neurotransmitters and their receptors in the rat SCG was investigated at light microscopical level using immunohistochemistry. The set of neurotransmitters was identified by examining transmitter-related enzymes and their transporters while the transmitter corresponding receptors were determined by applying subtype-specific antibodies. Immunohistochemistry revealed that ganglion neurons exhibited immunoreactivity for several biogenic amines. In particular, all the sympathetic neurons were immunostained for tyrosine hydroxylase (TH), a rate-limiting enzyme of catecholamine synthesis. Most TH-immunoreactive cells also contained histidine decarboxylase, the histamine-synthesizing enzyme, and some of them contained tryptophan hydroxylase, a key enzyme in the biosynthesis of serotonin. In addition, we were able to find that SCG neurons were richly endowed with the D2 dopamine receptor, 5-HT3 and 5-HT4 serotonin receptors, H1 and H3 histamine receptors, and P2X2 and P2X3 purinoceptors. It can be inferred that different transmitter systems, including dopaminergic, serotoninergic, histaminergic and purinergic, are involved in the sympathetic innervation of the CB, which consequently influence the chemoreceptor activity.

Key words: carotid body, immunohistochemistry, rat, retrograde labeling, superior cervical ganglion

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Received: June 02, 2009
Revision received: June 16, 2009
Accepted: July o6, 2009

Introduction

The superior cervical ganglion (SCG) provides sympathetic innervation of the head and neck structures, including the carotid body (CB). The ganglion is composed of functional subsets of neurons showing different sympathetic phenotypes [1]. In the rat these neuronal groups include secreto-, pilo-, and vasomotor neurons that selectively innervate different target organs. It is known that sympathetic vasomotor pathways play a major role in the control of peripheral vascular resistance in response to changing physiological demands [2].

The CB is the main peripheral chemoreceptor that senses the arterial blood levels of pO_2, pCO_2 and pH [3] and participates in the ventilatory responses to hypoxia,

hypercapnia and acidosis. Its principal cell type, type I (or glomus) cells, are dually innervated by both sensory nerve fibers of primary afferent neurons located in the petrosal ganglion and by postganglionic sympathetic nerve fibers from the SCG [4, 5]. The glomus cells share the same embryonic origin with the sympathetic neurons and are considered chemoreceptor elements within the CB [3, 4]. Sympathetic neurons of the SCG release neurotransmitters, which can postsynaptically activate the glomus cells, thus modulating the transmission of the chemosensory information.

Therefore, in the present study, we set out to investigate, by using immunohistochemical and retrograde tracing experiments, the presence of putative neurotransmitters and their corresponding receptors in the SCG that have been suggested to play a modulatory role in chemosensory function.

Materials and Methods

The experiments were carried out on Sprague-Dawley rats of both sexes (300 g body weight). All procedures were approved by the Animal Care and Use Committee of the Technical University Munich and were consonant with the ethical guidelines established by the National Institute of Health.

To identify the SCG cell bodies innervating the CB glomus cells we used retrograde neuronal labeling with a fluorescent retrograde tracer, Fast Blue (FB; Sigma, St Louis, MO, USA). The animals were anaesthetized with 4% chloral hydrate (1ml/100 g body weight, i.p.) and the bifurcation area of the common carotid artery was carefully exposed. A 2% aqueous solution of FB (10 µl) was slowly injected into the CB with a Hamilton microsyringe, and care was taken to prevent any leakage of the tracer from the site of injection by placing a plastic film around the CB. The film was left in place for the entire survival period. The animals were allowed to recover for 7 days. Thereafter, they were deeply re-anesthetized with chloral hydrate and perfused through the ascending aorta first with cold PBS, followed by 4% paraformaldehyde (PFA) in 0.01 M phosphate-buffered saline (PBS; pH 7.4). After perfusion, SCGs were quickly removed and postfixed in the same fixative for 1 h at 4ºC. 12 µm thick serial sections were cut on a freezing microtome, mounted onto uncoated slides and coverslipped with Vectashield (Vector Laboratories, Burlingame, CA, USA). The sections were examined with a Zeiss fluorescence microscope at 360 nm excitation wavelength (filter system A), which elicits the blue FB fluorescent labeling of the neuronal cell bodies.

For immunohistochemical experiments, the animals were transcardially perfused, first with 100 ml of heparinized (1 U/ml) PBS, followed by 500 ml of PFA in PBS. The carotid bifurcations with SCGs were dissected out and postfixed in the same fixative overnight at 4ºC. Subsequently, the tissues were embedded in paraffin and cut into 5 µm thick sections. The sections were then deparaffinized with xylene and isopropanol, and processed for avidin-biotin-horseradish peroxidase complex (ABC) immunohistochemistry. Briefly, the endogenous peroxidase was blocked with 1.2% hydrogen peroxide in absolute methanol, followed by antigen retrieval in 10 nm citrate buffer (pH 6.0) for up to 30 min in a microwave oven. After washing in PBS, the sections were preincubated for 30 min at room temperature in 5% normal goat serum to avoid nonspecific staining. They were then incubated in a humid chamber overnight at 4ºC with primary polyclonal antibodies against dopamine D2 receptor (D2R; BIOTREND Chemikalien GmbH, Köln, Germany), serotonin transporter (SERT; Alpha Diagnostic Int., San Antonio, TX, USA), serotonin 5-HT3 receptor (5-HT3R; Calbiochem, San Diego, CA, USA), serotonin 5-HT4 receptor (5-HT4R; Acris Antibodies GmbH, Hiddenhausen, Germany), histidine decarboxylase (HDC; Progen Biotechnik GmbH, Heidelberg, Germany), histamine 1 receptor (H1R; Acris), histamine 2 receptor (H2R; Alpha Diagnostic), 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, USA), P2X2 and P2X3 receptors (both from Abcam), all raised in rabbits and with mouse monoclonal antibodies to tyrosine hydroxylase (TX; LOXO GmbH, Dossenheim, Germany) and tryptophan hydroxylase (TPH; Calbiochem). After rinsing in PBS, the sections were reacted for 2h at room temperature 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 for 3-5 min with the chromogens DAB (Sigma) or Vector SG as peroxidase substrates, 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 details previously [6].

Results

The SCG is located at the bifurcation of the common carotid artery, near the CB (Figure 1A). To determine the location of the perikarya of sympathetic neurons linked to the glomus cells in the SCG, we first retrogradely labeled them with the tracer FB. Single FB-labeled neurons of various cell sizes were located predominantly in the caudal portion of the ganglion (Figure 1B).

In order to demonstrate the presence of putative neurotransmitters and their receptors in the SCG, immunostaining for the corresponding biosynthetic enzymes and transporters as well as the receptor subtypes was performed. Virtually all sympathetic neurons in the SCG contained TH, the rate-limiting enzyme of catecholamine synthesis (Figure 2A). Likewise, the vast majority of SCG neurons were also immunoreactive for VMAT1 (Figure 2B), transporting catecholamines, and were richly endowed with dopamine D2 receptors (Figure 1C).

Conversely, serotoninergic traits (TPH, a key enzyme in serotonin biosynthesis, and serotonin transporter), were only detected in a subset of SCG sympathetic neurons (Figure 3A, B). We also found that a few principal ganglion cells, primarily restricted to the caudal pole of SCG, expressed serotonin 5-HT3 and 5-HT4 receptors (Figure 3C, D).

Immunohistochemistry revealed that most of sympathetic neurons in the SCG were immunostained for HDC, the histamine-synthesizing enzyme, and for VMAT2, which is highly specific transporter for histamine (Figure 4A, B). In addition, a large number of sympathetic ganglion cells showed immunoreactivity for histamine H1 (Figure 4C) and H3 (Figure 4D) receptors, but not for H2 and H4 histamine receptors (not shown).

Using antibodies directed against P2X receptor subtypes, we identified that a number of SCG neurons were immunostained for the P2X2 (Figure 5A) and P2X3 (Figure 5B) receptors. The immunoreactive cells were dispersed throughout the ganglion with no apparent differences in their staining intensity.

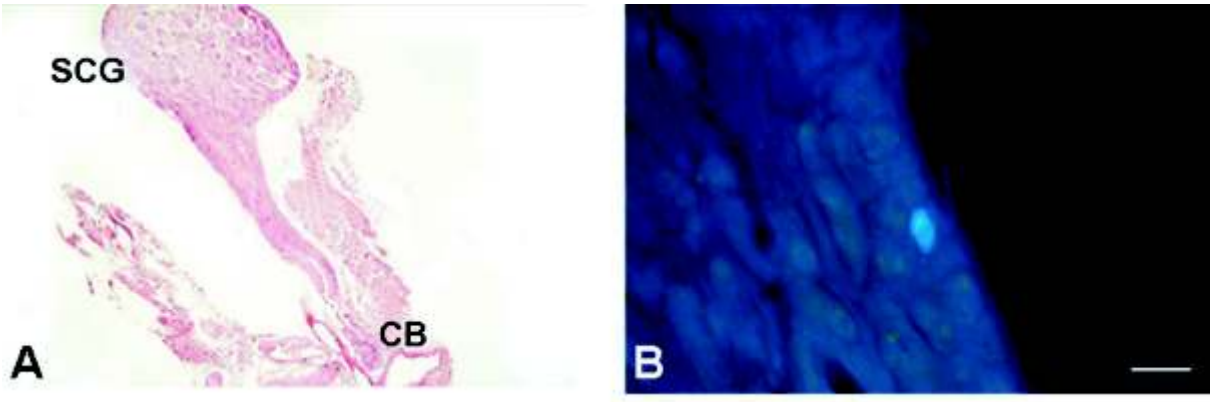

Figure 1. Superior cervical ganglion (SCG) of the rat. (A) A H&E-stained section showing the location of the ganglion near the carotid body (CB) at the bifurcation of the common carotid artery. (B) FB-labeled ganglion neuron profiles in the caudal portion of the SCG. Scale bar, 100 μm (A) and 50 μm (B).

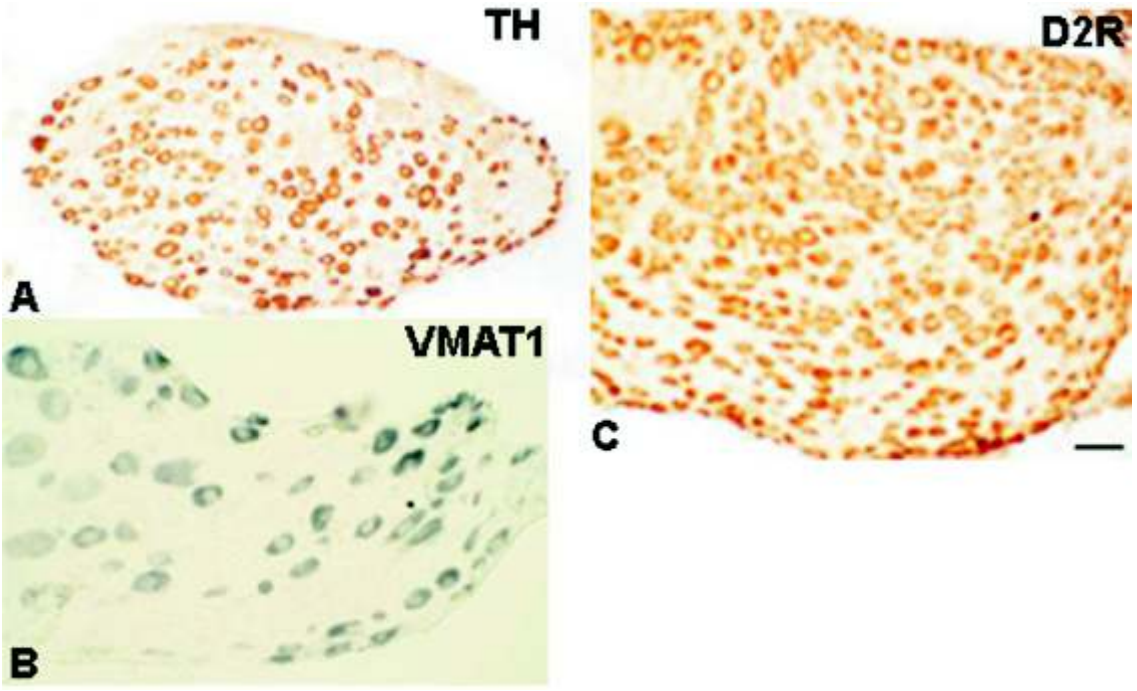

Figure 2. Dopaminergic traits in the rat SCG. (A) Virtually all sympathetic neurons are immunostained for tyrosine hydroxylase (TH). (B) shows the caudal pole of the ganglion with a large number of VMAT1-immunoreactive neuronal perikarya. Almost all SCG cell bodies express dopamine D2 receptors (C). Scale bar, 100 µm (A, C) and 50 µm (B).

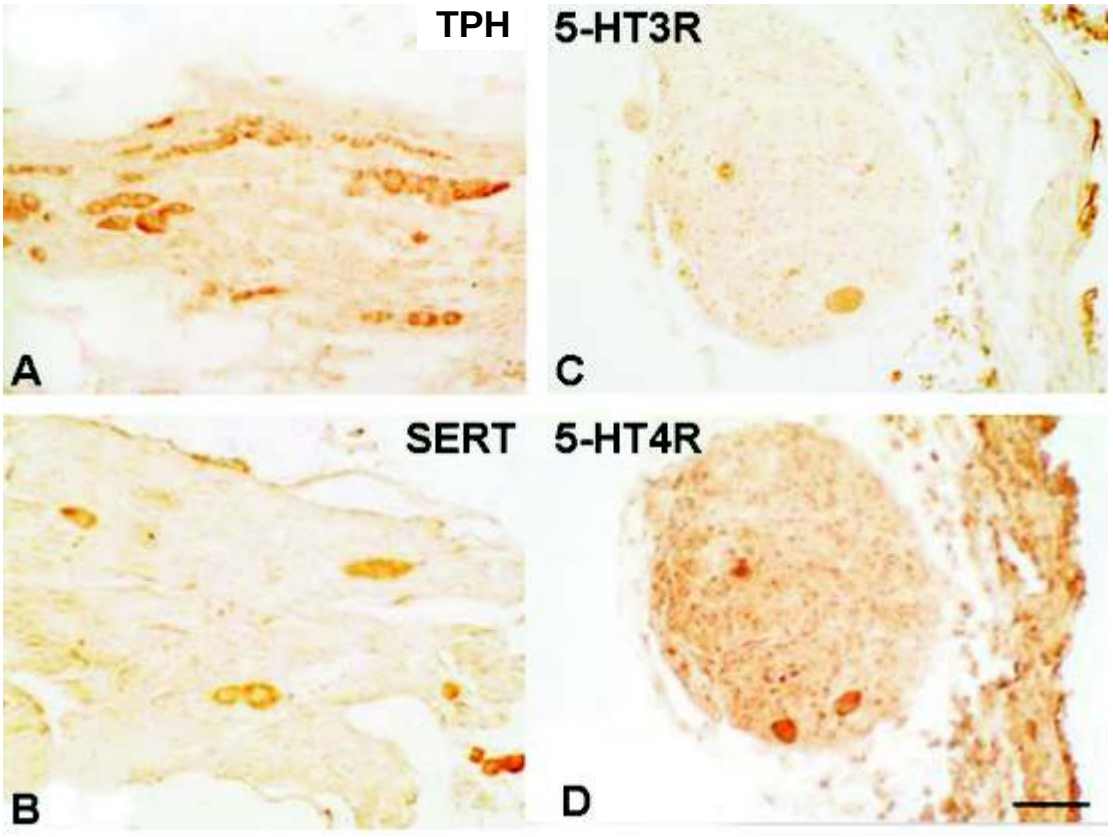

Figure 3. Serotoninergic traits in the rat SCG. (A) A subset of sympathetic neurons shows immunoreactivity for the serotonin-synthesizing enzyme tryptophan hydroxylase (TPH) and some of them contain its transporter SERT (B). Note that only a few SCG cells are immunoreactive for 5-HT3 (C) and 5-HT4 (D) serotonin receptors. Scale bar, 50 µm.

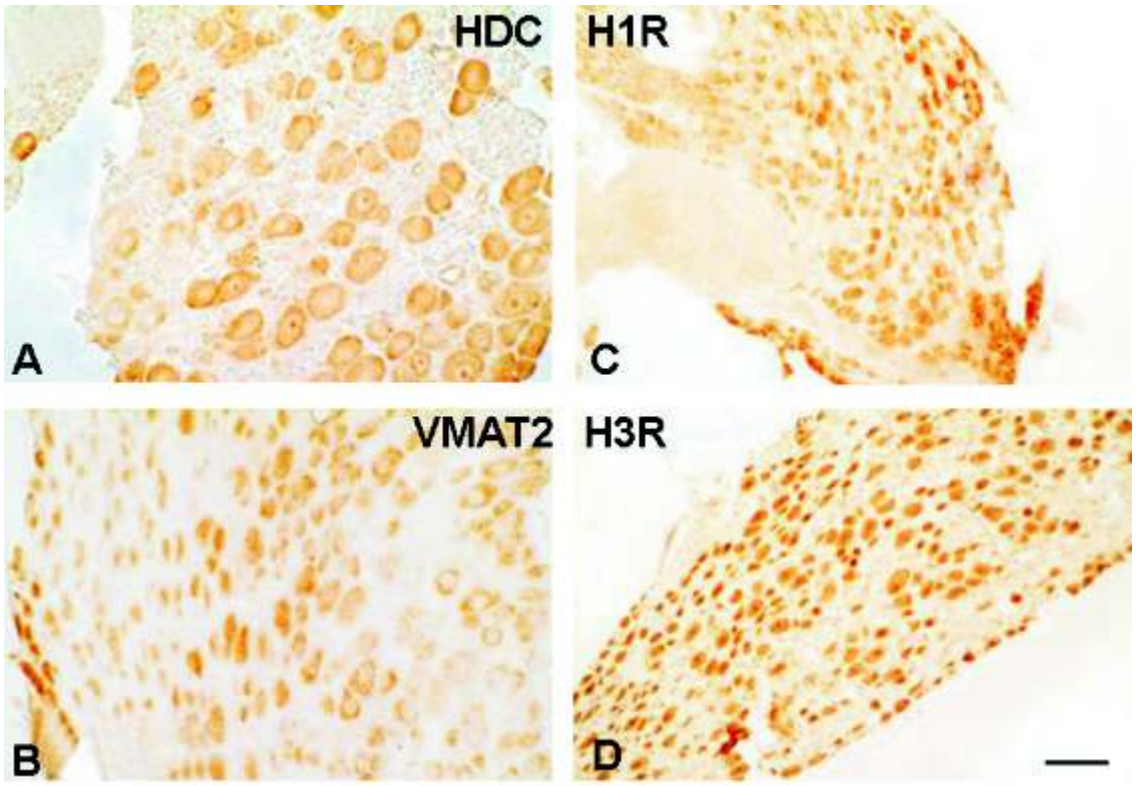

Figure 4. Histaminergic traits in the rat SCG. (A) Numerous histidine decarboxylase (HDC)-containing perikarya are visible in the caudal portion of the ganglion. Most of them exhibit strong immunoreactivity also for VMAT2 (B) and are richly endowed with histamine H1 (C) and H3 (D) receptors. Scale bar, 50 µm (A, B) and 100 µm (C, D).

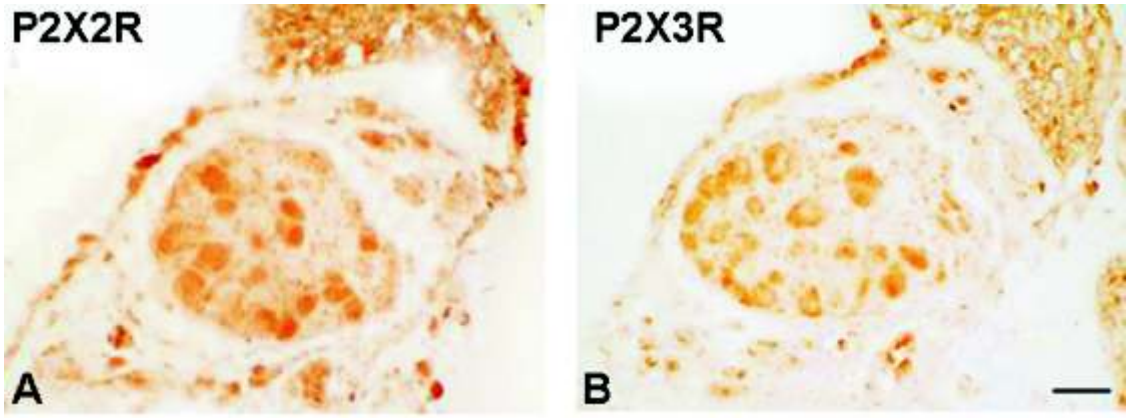

Figure 5. Purinergic receptors in the rat SCG. (A) Expression of P2X2 receptor in a subset of caudally located principal ganglion cells. (B) A number of perikarya in the distal SCG are immunoreacted with the P2X3 antibody. Scale bar, 50 µm.

Discussion

The results of the current study show that a subpopulation of neurons in the rat SCG expresses all the biochemical components for biosynthesis, storage and release of biogenic amines such as dopamine, serotonin and histamine, and are endowed with certain specific receptors at the presynaptic and/or postsynaptic levels.

It is noteworthy that virtually all sympathetic neurons contain TH (see also [5]), its components of exocytotic apparatus and D2 dopamine receptors. Our present findings allow for more

definitive characterization of dopaminergic cells involved in hypoxic chemosensitivity modulation. It is likely that D2 receptors serve as presynaptic inhibitory autoreceptors of dopaminergic glomus cells [7] and may function as postsynaptic receptors of SCG sympathetic neurons as well [8].

Our observations also indicate that a subset of caudally located SCG sympathetic neurons possesses serotoninergic traits, i.e. contains the serotonin-synthesizing enzyme TPH, its transporter SERT and expresses 5-HT3 and 5-HT4 receptors. Serotonin was recently found in rodent glomus cells to act as a modulator of their chemoreceptor function [9] although the cardinal property, its release in response to hypoxia by the CB, has not been observed [10]. Thus, the role of serotonin as a primary transmitter in hypoxic chemosensitivity has yet to be confirmed.

On the other hand, our prior experiments suggested that histamine is synthesized, stored and released during hypoxia from the glomus cells in the CB of rats [6,11]. Here we observed that histaminergic traits (HDC and VMAT2) appear to be expressed in most sympathetic neurons of rat SCG as well. Together the results confirm earlier evidence that virtually all vasomotor neurons in the rat SCG express HDC [12] and VMAT2, while VMAT2-negative neurons are likely to have secretomotor and pilomotor phenotypes [13]. Moreover we show that histamine H1 and H3 receptors are present in almost all sympathetic ganglion cells. Through this we confirm the recent data of Cannon et al. [14] that the SCG may contain histamine H1R- and H3R-immunopositive neurons that innervate structures other than the skin. It is known that activation of H1 receptors facilitates release of neurotransmitters whereas H3 receptor activation results in depressed release [15]. In other words, this amine may also produce inhibitory autocrine-paracrine modulation of the chemosensory function [16].

In the rat CB, ATP is a key transmitter released by chemoreceptor cells acting via ionotropic P2X receptors [17]. It has been suggested that the hyperpolarization of sympathetic ganglion cells produced by catecholamines may result from the elevation of intraneuronal cyclic AMP [18]. All preceding evidence indicates that ATP is released from the rat SCG and that this release can be presynaptically modulated by various receptors [19]. Xiang et al. [20] detected immunoreactivity for P2X1-4 and P2X6 receptors in SCG of the rat. As a consequence, ATP has a variety of effects, hyperpolarizing rat CB [21] and SCG [22].

Conclusion

In conclusion, most glomus cells are under an inhibitory control due to sympathetic influence from the SCG. Specifically, different transmitter systems are involved in the sympathetic innervation of the CB, and consequently influence its chemoreceptor activity. These include biogenic amines like dopamine, serotonin and histamine as well as adenosine nucleotides such as ATP. It seems that chemoreceptor transduction and transmission mechanisms may be differentially modulated through inhibitory receptors leading to a negative feedback regulation of the neurotransmitter release during chemosensory signaling.

Acknowledgements

This work was supported by the Alexander von Humboldt Foundation to N. Lazarov through the resumption of a fellowship (grant 1015945). S. Reindl was supported in the Graduate School 333 (Biology of human diseases) by a predoctoral fellowship of the Deutsche Forschungsgemeinschaft. We thank Marlies Rauchfuß and Barbara Zschiesche for their excellent technical assistance.

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