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Expression of Some Neuropeptides in the Rat Carotid Body

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The carotid body (CB) is a polymodal oxygen sensor strategically positioned at the bifurcation of the common carotid artery and endowed with the ability to detect broadly the chemical composition of arterial blood. The chemoreceptor glomus cells of the carotid body work in concert with the opposing afferent nerve endings of the petrosal ganglion cells and together they form a functional unit. Evidence to date suggests that the chemoreceptor cells utilize various chemical substances for the afferent transmission of chemosensory information. The presence and distribution of substance P, neuropeptide Y and methionine-enkephalin immunoreactive nerve structures in the CB of rats were identified at a light microscopical level using immunohistochemistry. All the three examined neuropeptides were expressed in both the glomus cells and nerve fibers, albeit in a different manner. Our results provide immunohistochemical evidence that the rat CB utilizes sensory and autonomic peptides, which can modulate chemosensory activity through their actions on the glomus cells and vasculature.

Key words: carotid body, chemosensitivity, immunohistochemistry, neuropeptides, rat

Introduction

The carotid body (CB) is the main peripheral chemoreceptor responsible for monitoring changes in pO2, pCO2 and pH in arterial blood and participates in the ventilatory responses to hypoxia, hypercapnia and acidosis [5]. It is bilaterally located at the carotid bifurcation, between the external and internal carotid arteries. From a structural point of view, the organ is composed of lobules, which are organized in clusters of cells, surrounded by a dense meshwork of capillaries and penetrated by bundles of sensory nerve endings of the carotid sinus nerve, a branch of the glossopharyngeal nerve as well as by postganglionic sympathetic nerve fibers from the superior cervical ganglion [5].

The cell clusters, also known as glomeruli, consist of two major cell types: neurosecretory oxygen sensitive type I or glomus cells, which are round in shape and glial type II or sustentacular cells, which are fusiform and located at the periphery of the clusters. Type II cells mainly play a supportive role, but may also behave as stem cell precursors for type I cells [12]. Sustentacular cells have been suggested to co-ordinate chemosensory transduction through interactions with nerve endings, type I cells and blood vessels [14]

It has been proposed that the glomus cells in different animal species release a variety of transmitter candidates including classical and peptide transmitters. Previous studies have shown that the chemoreceptor cells contain diverse neuropeptides. Among them are sensory peptides such as substance P (SP) [7], autonomic neuropeptides like neuropeptide Y (NPY) [9] and the opioid peptide met-Enkephalin (mEnk) [17].

Materials and Methods

The experiments were carried out on adult Wistar rats of both sexes, weighing 250-300 g. All procedures were performed according to a standard protocol established by the Bioethical Commission of the Biomedical Research at the Institute of Neurobiology of the Bulgarian Academy of Sciences. All efforts were made to minimize the number of animals used and their suffering.

For the immunohistochemical experiments, the rats were deeply anesthetized and transcardially perfused first with 0.05 M phosphate-buffered saline (PBS), pH 7.3, followed by 4% paraformaldehyde (PFA) in 0.01 M phosphate buffer (PB), pH 7.3. 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 7 μm thick sections. The samples were then deparaffinized with xylene and ethanol, and subsequently processed for avidin-biotin-horseradish peroxidase complex (ABC) immunohistochemistry. Briefly, the sections were treated with hydrogen peroxide (1.2% in absolute methanol; 30 min) to inactivate endogenous peroxidase and the background staining was blocked with 5% normal goat serum (NGS) in PBS for 1 hour. Between the separate steps, the sections were rinsed with cold PBS/Triton X-100. Afterward, they were incubated for 24 h at room temperature with the respective primary antibodies, rabbit anti-SP (diluted 1:1000, Abcam, Cambridge, UK), rabbit anti-NPY (1:500; Amersham International, Buckinghamshire, UK) and rabbit anti-mEnk (1:1000, Inc Star, Stillwater, MN, USA) overnight at 4°C in a humid chamber, followed by biotinylated goat anti-rabbit IgG (Sigma, 1:250) for 2 h at room temperature, and lastly the ABC complex (Vector Labs, Burlingame, CA, USA) was applied for 2 h at room temperature. Finally, the peroxidase activity was visualized using diaminobenzidine as a chromogen. After the immunoreaction, the sections were dehydrated in ethanols, cleared in xylene and coverslipped with Entellan (Merck, Darmstadt, Germany). The slides were observed and photographed with a Nikon research microscope equipped with a digital camera DXM1200c.

The specificity of the immunostaining was controlled by omission of the primary antiserum from the incubation medium or its replacement by PBS. No immunoreactivity was detected in either case.

Results

A dense plexus of substance P-immunopositive intraglomerular and periglomerular nerve fibers were observed throughout the CB and many of the varicose fibers were closely associated with glomus cells (Fig. 1A). At a higher magnification, a large number of SP-immunostained glomus cells were also seen within the CB (Fig. 1B).

In addition, abundant NPY-like immunoreactive nerve fibers were found throughout the parenchyma of the adult rat CB (Fig. 1C). The nerve fibers appeared as varicosities and most of them were associated with small vessels. On the other hand, only scattered glomus cells were NPY immunostained.

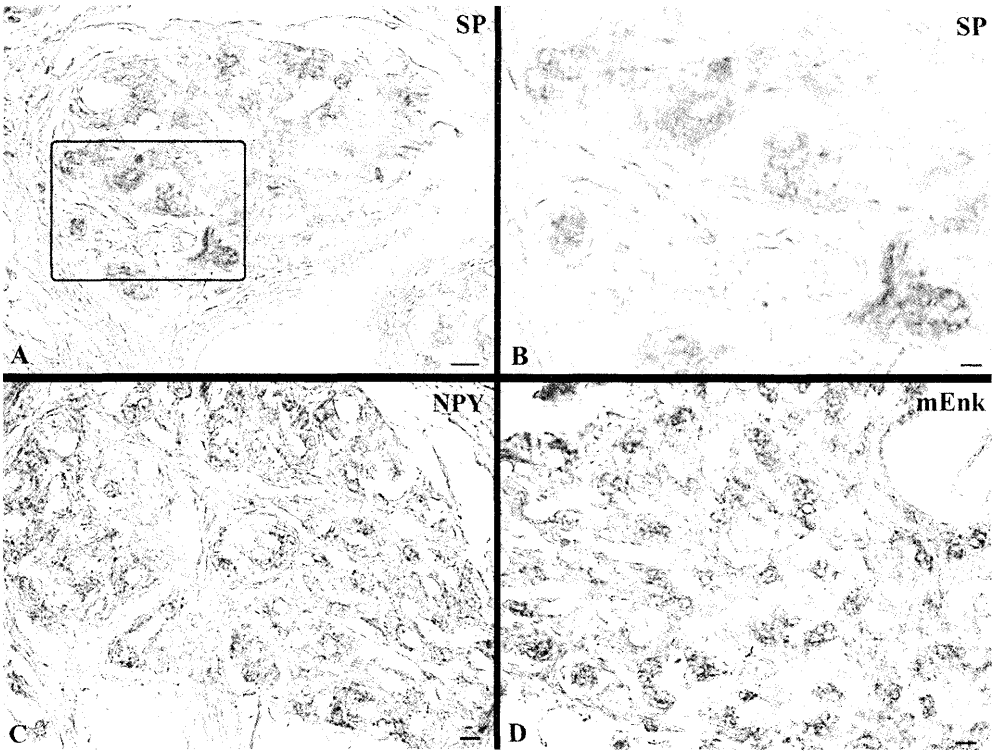

Fig. 1. (**A**) SP-immunoreactive intaglomerular and periglomerular nerve fibers in the rat CB. (**B**) A higher magnification of the boxed area in (A) showing a large number of SP-expressing glomus cells (**C**) NPY-immunoreactive perivascular nerve fibers and scattered immunostained glomus cells in the adult rat CB. (**D**) mEnk immunopositive glomus cells and nerve fibers. Scale bars = 25 μm (**A, C, D**); 50 μm (**B**).

Conversely, the immunohistochemical experiments demonstrated immunostaining for mEnk in the majority of glomus cells (Fig. 1D). Intraglomerular and periglomerular nerve fibers within and around the cell clusters were mEnk-immunostained as well.

Discussion

The present results demonstrate that all the three examined neuropeptides are expressed in the rat CB, albeit in a different manner. In this study, we find that the vast majority of glomus cells appear to contain certain sensory peptides like SP and mEnk. Moreover, the most numerous SP-immunoreactive fibers are associated with the glomus cells, though some immunostained varicosities were seen periglomerularly too. Hence, we have found SP immunoreactivity in both the glomus cells and nerve fibers in the rat CB in contrast with studies performed by other authors [2,7,17] which have been reported that SP immunoreactivity is only localized to the nerve fibers within the CB of this species. Furthermore, SP immunoreactivity has been found in the CBs from diverse animal species, although its specific cellular distribution to either carotid sinus nerve fibers or glomus cells appears to be species dependent. Indeed, SP has been reported to occur

in glomus cells of the human CB [13] or only in the glomic nerve fibers of this species [10]. Immunofluorescent studies [3] have also shown that SP is located in a few glomus cells and in nerve fibers present on the cat CB, while all previous studies have reported SP immunoreactivity exclusively in the nerve fibers within the rabbit CB [8]. These results suggest that SP is involved in chemosensory mechanisms in the CB of several animal species, including the rat CB. Taken together with previous immunohistochemical [7,17] and physiological [4] reports, our data indicate that SP probably modulates the CB chemosensitivity and indirectly increases carotid sinus nerve activity.

Our immunohistochemical data on the strong expression of mEnk immunoreactivity in both the glomus cells and nerve fibers innervating the CB of the rat are in contrast with the immunohistochemical findings of Heym and Kummer [6] who reported mEnk immunoreactivity only in the nerve fibers. Physiologically, it is likely that mEnk inhibits the sensory activity of the CB.

The present findings on the strong expression of NPY immunoreactivity in the perivascular nerve fibers and weak NPY-like immunoreactivity in the adult rat CB are in accordance with the immunocytochemical data of Oomory et al. [11]. These authors have reported that NPY immunoreactive glomus cells decrease rapidly from the first to the second postnatal week. On the other hand, NPY immunostained nerve fibers mainly increase after the second postnatal week [11]. From a functional point of view, as an autonomic neuroactive peptide, NPY possibly leads to the CB excitation by causing local vasoconstriction.

In conclusion, both excitatory and inhibitory neuropeptides are involved in the modulation of sensory response of the rat CB.

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