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Lectin and Peptide Expression in Nodose, Sphenopalatine and Superior Cervical Ganglia of The Rat

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Abstract: The presence and distribution of *Griffonia Simplicifolia* I-B4 (GSA I-B4) and Calcitonin Gene Related Peptide (CGRP) were studied in the nodose ganglion (NG) (inferior ganglion of the vagus nerve), sphenopalatine ganglion (SPG) and superior cervical ganglion (SCG) of the rat. GSA I-B4 labeling was found in all ganglia tested. Neither SPG nor SCG cell bodies stained with CGRP were undetectable. The pattern of the distribution of GSA I-B4 and CGRP labeled cells were quite similar in the nodose ganglion. They were found in the poles of the ganglion with some marginal labeling. Large numbers of GSA I-B4 and CGRP labeled cells were found and the number of labeled cells did not vary considerably between the two markers in this ganglion. GSA I-B4 labeled neurons of the SPG and SCG were fewer in numbers compared with NG.

These data demonstrate the presence of a “non-peptide” population of unmyelinated primary afferents in sensory and autonomic ganglia with the lack of CGRP immunoreactivity in the autonomic ganglia. This suggests that the “non-peptide” group of primary afferents are involved in different functional mechanisms than peptidergic afferents.

Key Words: lectin, calcitonin gene-related peptide, rat, autonomic and sensory ganglia

Rat'ın Nodos, Sphenopalatine ve Superior Cervical Ganglion'larında

Lectin ve Peptit Varlığının İncelenmesi

Özet: *Griffonia Simplicifolia* I-B4 (GSA I-B4) ve Calcitonin gene related peptide (CGRP)'nin varlığı ve dağılımı rat'ın nodos ganglion'unda (NG) (nervus vagus'un inferior ganglionu), sphenopalatine ganglion'unda (SPG) ve superior cervical ganglion'unda (SCG) çalışıldı. GSA I-B4 test edilen üç ganglion'da da bulundu. CGRP immunoreaktiv hücelere ne SPG'de nede SCG'de rastlandı. Nodos ganglion'da GSA I-B4 ve CGRP immunoreaktiv hücelerin dağılımı örneği oldukça bir birine benzerdi. Her iki marker'a bu ganglion'un kutuplarında ve az sayıda da kenarlarında rastlandı. Nodos ganglion'da çok sayıda GSA I-B4 ve CGRP immunoreaktiv hücelere bulundu ve bu markerların sayıladığı arasında çok bir farklılık gözlenmedi. GSA I-B4 pozitif hücelere SPG ve SCG'de sayı olarak NG'de de bulunanlardan daha azdı.

Bu bulgular, “non-peptide” miyelinsiz primer afferentlerin varlığını sensorik ve otonomik ganglion'larda gösterdi. Fakat CGRP'nin varlığı otonomik ganglion'larda gözlenemedi. Sonuç olarak, bu bulgular “non-peptide” primer afferent sinir hücelerinin peptidergic afferent sinir hücelerinde farklı bir mekanizmada fonksiyon yaptığını akla getirir.

Anahtar Sözcükler: lectin, calcitonin gene-related peptide, rat, otonomik ve sensorik ganglionlar.

Introduction

The vagal nerve includes sensory neurons which relay information from the viscera to the nucleus of solitary tract (NTS), in addition to autonomic and motor neurons. Cell bodies of the majority of sensory fibers are in the nodose ganglion. Sensory neurons from the nasal and palatal mucosa traverse from the SPG to the CNS via trigeminal and facial nerves. Parasympathetic fibers from the lachrymal nucleus synapse with postganglionic

parasympathetic fibers in sphenopalatine ganglion. The postganglionic fibers reach the lachrymal gland and mucose glands in the mucosa that lines the nasal cavity and paranasal sinuses. Postganglionic sympathetic fibers from the SCG are distributed to the blood vessels, erector pili and sweat glands of the head. The postganglionic sympathetic fibers of the internal carotid nerve and pterygoid nerve also traverse from the SPG and distribute to the nasal and palatal mucosa (1-2).

Plant lectins are constituted from proteins or glycoproteins which bind to carbohydrate sites on cell membranes (3). Isolectin GSA I-B4 from *Griffonia simplicifolia* (*Bandeireae simplicifolia*) (4) has been found to selectively bind to a subpopulation of small diameter primary afferents in dorsal root ganglia (5-9), most of which are positive for fluoride-resistant acid phosphates (FRAP) (7), and in the other sensory ganglia (10-13) including autonomic sphenopalatine ganglion (12). It has been demonstrated that GSA I-B4-reactive cells constitute a separate population of small diameter primary afferents from those constituted by CGRP and Substance P (SP) (10). Therefore it is an essential marker for the non-peptide group of C-fibers primary afferents (12, 13). CGRP immunoreactive somata have been shown in the nodose and sphenopalatine ganglia (14-16). It has been also shown that CGRP sensory fibers originating in the palatal and nasal mucosa as well as in the orbita traverse from the SPG via trigeminal (maxillary) and facial (pterygoid) nerves (14). In the present study, the presence and distribution of peptidergic and non-peptidergic neurons were studied in the nodose, sphenopalatine and superior cervical ganglia by GSA I-B4 labeling and CGRP immunoreactivity.

Materials and Methods

Tissue preparation:

Experiments were performed on nine adult Wistar rats of either sex (250-450 g body weight). Animals were deeply anesthetized with sodium pentobarbitone (50 mg/kg, I.P.), heparinized (1000 U injected intracardially) and perfused transcardially with 150 ml phosphate-buffered saline (PBS). Then, they were fixed by 300 ml of 4 % paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). The nodose and superior cervical ganglia were dissected by exposing the region of the cervical vagosympathetic trunk and tracing the its division cranially into the NG and SCG. The sphenopalatine ganglion was dissected by exposing the trigeminal nerve trunk which was retracted dorsally to make visible the SPG on the dorsal surface of the maxillary bone. Then, ganglia were removed and post-fixed for 2 h in the same fixative to that used in the perfusion. Tissue samples were cryoprotected with 20 % sucrose in PBS overnight. Then, transverse sections of the ganglia were serially cut with a cryostat at 10 μ m and thaw-mounted onto chrome-alum-gelatin-coated slides. The sections were air-dried for 2 h prior to staining.

Lectin histochemistry and immunohistochemistry:

Sections were washed in PBS (4 changes 15 minutes intervals) in a glass which was placed on a shaker (Jencons Scientific Ltd.). After washing, a ring was scored on the glass slide with a diamond marker around the section to serve as a barrier to the flow of antisera. Then, sections were covered with drops of biotinylated GSA I-B4 antisera (5-10 mg/ml, Sigma) in PBS containing 2.5 % bovine serum albumin (BSA) and 0.1 % Tritone X-100 overnight at 4°C. Thereafter, sections were washed in PBS (4 changes 15 minutes intervals) and incubated with Streptavidine HRP for 1 h at room temperature. To reveal the presence and distribution of HRP, glucose oxides, nickel and diaminobenzidine chromogene of Shu et al. (17) was used. Following the staining, sections were washed in PBS and distilled water, then air-dried, dehydrated and covered with cover slides via DPX.

Immunohistochemistry for CGRP was carried out using the same protocols to those used for the GSA I-B4 histochemistry. The method was only modified for biotinylation. In this case, following the incubation with the CGRP primary antisera (1:1000, a gift from Dr. P.K. Mulderry) the second step incubation was with biotinylated antisera for 1 h at room temperature.

Control experiments were carried out by pre-incubating the lectin with 0.1 M D-galactose (Sigma) which eliminated the staining. Absorption control was also used for the peptide antibody. Omission of the primary antibody was used in assessing the background staining levels.

Ganglia cell counting was performed by alternatively staining with Cresyl Violet. Cell counting was made on sample sections at approximately 100 μ m intervals through each ganglion. Only cells having clear nuclei were counted in the sections.

Results

GSA I-B4 was found in neurons of NG, SPG and SCG. Approximately half of the total population of neurons in nodose ganglion (49.4, 43.1, 45.1, 40.8 % in four different rats) were intensively stained GSA I-B4. Lectin binding was found in the cytoplasm of small neurons (Fig.1). Distribution of the lectin was generally polar and marginal in the NG (Fig.2). Bundles of GSA I-B4-reactive fibers were found to be traversing at the center of the NG. The polar and marginal distribution of the lectin was also present in the SPG (Fig.3) and SCG. In addition, numerous lectin-labeled axons were also observed in SPG and SCG. Lectin-reactive cells were less than 10 % of the

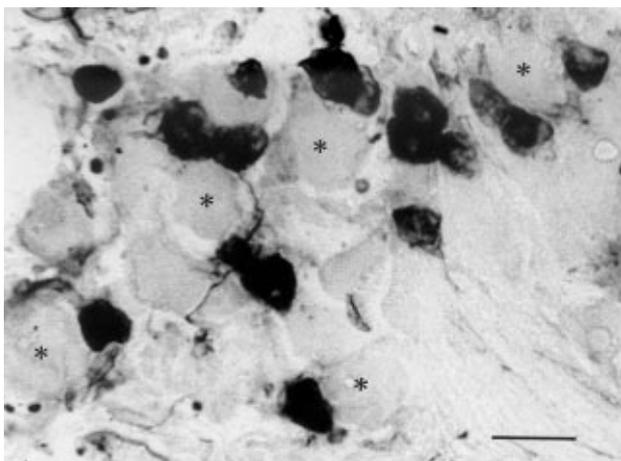


Figure 1. High magnification of small neurons stained with the GSA I-B4 lectin in section of nodose ganglion. Lectin-positive small primary sensory neurons exhibit intensive cytoplasmic reactivity, while large neurons are lectin-negative (stars). Scale bar: 100 μ

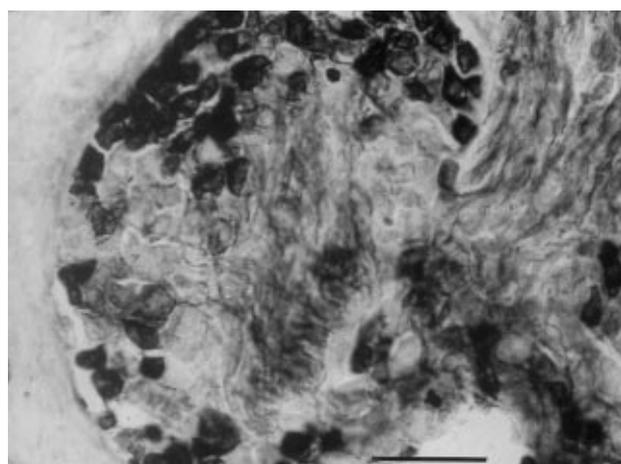


Figure 2. Low magnification of neurons labeled by GSA I-B4 in nodose ganglion. The vast majority of neurons are labeled by the lectin in margins of the ganglion. A course of lectin labeled fibres are seen in the middle of the ganglion. Scale bar: 100 μ

total counted cells of SCG (9.5, 4.4 %, in two rats). The figures obtained from SPG (6.7, 11.2 %, in two rats) were not very different from those obtained from SCG. The relative number of lectin-labeled cells counted in these ganglia were NG>SPG>SCG.

CGRP immunoreactivity was found in the neuron somata of the NG but in the axons of the SCG and SPG. No CGRP immunoreactivity was found in the cell bodies of the SCG and SPG. Distribution of CGRP immunoreactivity in NG was found to be similar to that seen with GSA I-B4 in NG. The number of CGRP-labeled

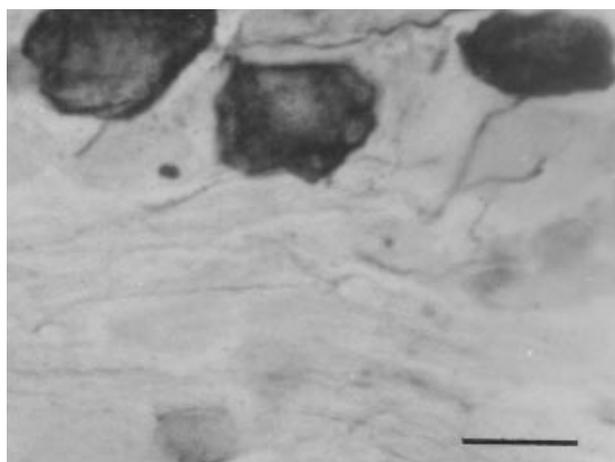


Figure 3. A few plasmalemmal and cytoplasmic lectin labeled cells in the margin of the sphenopalatine ganglion. Scale bar: 50 μ

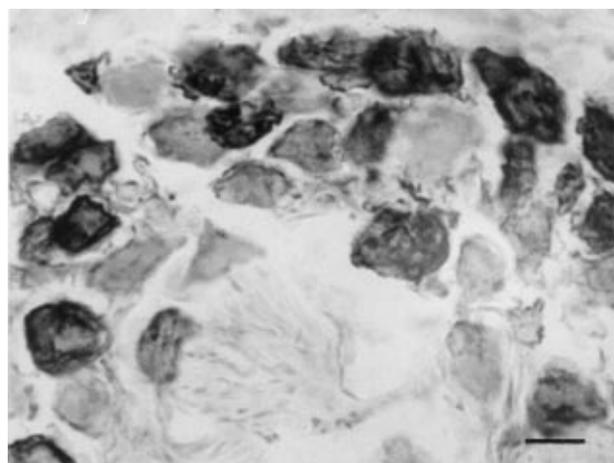


Figure 4. CGRP immunoreactive nodose ganglion cells. The intensity of labeling is weaker than lectin labeling in this ganglion. Scale bar: 800 μ

cells in this ganglion was also close to that of lectin-labeled cells in NG (50.89, 40.78, 40.00 %, in three rats). By comparison, CGRP immunoreactive labeling pattern was less intense than those labeled by the lectin in NG (Fig.4).

Discussion

Lectin-reactive neurons have been demonstrated in sensory and autonomic ganglia (5, 10-13, 18, 19). The data presented here confirm the finding that GSA I-B4-positive cell bodies present in NG and SCG (11, 12). The present data however demonstrate the presence of GSA I-B4-labeled cell bodies in SPG. Lack of lectin reactivity in neuron somata of SPG and other parasympathetic ganglia, such as otic and ciliary ganglia, has been reported

(12). In our study, GSA I-B4-positive neurons were found at the edges and poles of the SPG. Non-staining of GSA I-B4 in SPG found in the previous study could be due to the fixative (20) or fixative parameters (11, 13). Moreover, it has been reported that glial cells were more intensely stained with GSA I-B4 in purely-fixed tissue than those stained with GSA I-B4 in well-fixed tissue (13).

The presence of GSA I-B4 in neurons of NG indicates that these neurons contribute to the innervation of the nucleus of the solitary tract (NTS) and area postrema of the brain stem, the central projection sites for the general visceral afferent neurons of the vagus nerve. This has been confirmed by the demonstration that lectin-reactive axons terminate in the NTS and area postrema (11).

GSA I-B4-reactive neurons observed in SCG and SPG were fewer than those observed in NG. This indicates that these two autonomic ganglia largely rule out somatosensory innervation. Peptidergic neurons play a role in a modulatory interaction between the peripheral autonomic and sensory system (14). A small number of co-localizations of GSA I-B4 and neuropeptides have been reported in the nervous system (10, 12). In this way the lectin-positive neurons in these autonomic ganglia may be involved in the interaction. This needs further investigation. Despite the sensory function of the lectin-reactive neurons in the central nervous system (6, 21-24) their function in the peripheral nervous system remains unclear. Their expressions are not also limited to the neuronal cells. They are expressed by a variety of cell types (22, 25, 26). Due to their selective affinity for carbohydrate residues, lectins have been widely used for identifying the expression of glucoconjugates in the nervous system as well as other tissues. Isolectin GSA I-B4 binds specifically to a terminal α -D-galactose on cell

surface of a subpopulation of small diameter primary afferents and their terminals (5, 21, 6). Furthermore, it has been reported that the majority of peripheral unmyelinated somatosensory afferents are specifically labeled by lectins (12). In the present study, it was found that GSA I-B4-positive neurons were smaller than unlabeled neurons in NG. Hence, it may be suggested that lectin labeled neurons are sensory and could be reasonable candidates for nociceptive mechanisms in the periphery. However, reports such as "lectin-reactive neurons are not entirely sensory" (7, 22) should be taken into consideration. Our results also suggest that lectin-reactive neurons found in the autonomic ganglia have the same cell surface carbohydrate binding site as those found in the sensory ganglion.

CGRP immunoreactivity has been found in a subset of sensory ganglion cells (10, 22). These cells are believed to be nociceptive. They are larger in diameter than those lectin-labeled neurons (12). This indicates that CGRP and lectin-positive cells represent a different subpopulation of sensory neurons which probably take a role in different functional mechanisms. In an experiment, a few CGRP-positive cells have been found in SPG (16). No CGRP immunoreactive cell bodies have been reported in SCG (27). In our study, no CGRP immunoreactivity was observed either in SPG or in SCG. The reason for the presence of CGRP immunoreactivity in SPG found in the above study could be due to the colchicine treatment, which inhibits axonal transport and helps to visualize the neurotransmitters in the cell body, before being killed. In our study, the less intense staining of CGRP in the NG could be due to the decrease in the CGRP level. We found that the distribution and number of CGRP immunoreactive cells were similar to the GSA I-B4-labeled cells in NG.

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