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Distribution of non-peptidergic primary afferents in trigeminal ganglion of the rat

Zafer Soygüder Zekeriya Özüdoğru

Yüzüncü Yıl Üniversitesi, Veteriner Fakültesi, Anatomi Anabilim Dalı, Van, TÜRKİYE

Abstract: The distribution of isolectin I-B4 from *Griffonia Simplicifolia* (GSA I-B4) was studied in trigeminal ganglion. Small diameter cell bodies were labeled by GSA I-B4, while large cells were unlabeled. GSA I-B4-labeled axons from the maxillary, mandibular and ophthalmic branches of the trigeminal nerve reached to their cell bodies which were located in discrete regions within the ganglion. The distribution of non-peptidergic primary afferent fibres in trigeminal ganglion is briefly discussed with those obtained in other studies using GSA I-B4 as a marker in trigeminal ganglion.

Key Words: GSA I-B4, Lectin, Rat, Trigeminal ganglion.

Ratın trigeminal ganglionundaki non-peptidergic primer afferentlerin dağılımı

Özet: *Griffonia Simplicifolia* tohumlarındaki isolectin I-B4 (GSA I-B4)'un dağılımı ratın trigeminal ganglionunda çalışıldı. GSA I-B4 sadece küçük çaplı ganglion hücrelerinde gözlendi. Buna rağmen büyük çaplı hücrelerde bu lectinin varlığına rastlanmadı. Mandibular, maxillar ve ophthalmic GSA I-B4-positive axonların trigeminal ganglionda birbirinden bağımsız yerlerde yerleşen ganglion hücrelerine vardıkları gözlendi. Trigeminal ganglionda non-peptidergic primer afferent sinirlerin dağılımı, GSA I-B4'u trigeminal ganglionda marker olarak kullanan çalışmalarla kısaca tartışılacaktır.

Anahtar Kelimeler: GSA I-B4, Lectin, Rat, Trigeminal ganglion.

INTRODUCTION

Plant lectins (1, 2) have been widely used as a marker for unmyelinated primary afferents in central (3-11) and peripheral (3-10, 12-17) nervous system. Isolectin I-B4 from the seeds of *Griffonia Simplicifolia* (Bandeiraea Simplicifolia) (GSA I-B4) has been claimed as a good marker for non-peptide population of unmyelinated primary afferents (3, 4, 10, 15, 16) which are probably nociceptive and have smaller diameter than those containing peptides (16). The lectin co-localises with sensory neurone-specific acid phosphate isoenzyme, fluoride-resistant acid phosphate (FRAP) in sensory neurones and their peripheral terminals (12, 16). However, few co-localisation of the GSA I-B4 with peptides has been reported in dorsal root (16), trigeminal (3) and jugular ganglia (superior ganglion of vagus nerve) (10).

Trigeminal ganglion lies within the cranial cavity, embedded in the duramater near the cavernous sinuses (18). The peripheral processes of trigeminal ganglion cells constitute the ophthalmic and maxillary nerves and the sensory component of the mandibular nerve. These three branches of the trigeminal nerve arise from the ganglion and distribute to skin of the face and forehead, the scalp, the mucosa of the oral and nasal cavities, the paranasal sinuses, the teeth and the dura mater (19). The central processes of the trigeminal nerve terminate in the brain stem (19, 20).

In the present study, lectin expression by the trigeminal afferent fibres was studied histochemically, using the GSA I-B4, to examine the distribution of non-peptidergic primary afferents in the trigeminal ganglion.

MATERIALS AND METHODS

Four adult Wistar rats of either sex weighing 350 gr. were deeply anaesthetised with sodium pentobarbitone (50 mg/kg), heparinized (1000 U injected intracardially) and perfused through left ventricle first 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). Trigeminal ganglia were removed and postfixed for 2 hours in the same fixative that used in perfusion. Tissue samples were cryoprotected with 20 % sucrose in PBS overnight. Tissues were sectioned at 40μm thickness with a freezing microtome. Sections were collected into 0.1 M PBS and processed as free-floating sections. Then they were washed in PBS (four changes 15 minutes intervals) and incubated free-floating in biotinilated antisera to GSA I-B4 (5-10 mg/ml Sigma) in PBS containing 2.5% bovine serum albumin and 0.1% Triton X-100 overnight at 4 °C. The sections were then washed in PBS (four changes 15 minutes intervals) for removing unbound lectin and incubated with streptavidine HRP for 1 hour at room temperature. Sections were again washed and processed according to the chromogen protocol by Shu et al. (21).

Control experiments were done by the preincubation of the lectin with 0.1 M D-galactose, which eliminated the staining. Background staining levels were assessed by omitting of the primary antibody. Suppression of endogenous peroxidas was done by pre-treatment of the sections with 0.3 % H_2O_2 in PBS for 30 minutes before the histochemistry.

RESULTS

The cells binded with GSA I-B4 were small diameter neurones in the ganglion, while large neurones unlabeled with the lectin (Fig. 1). The lectin binding was intense in the cytoplasm around the nucleus. Labelled axons coursed into the ganglion and reached to their cell bodies located in discrete regions within the ganglion (Fig. 2). The regions in which the cell localised were poles of the ganglion. The lectin reactive axon bundles were intensely stained and could be traced back to the ganglion. Some axon bundles traversed through the ganglion without terminating at the lectin-reactive cell bodies.

DISCUSSION

Present results confirmed the previous finding that the isolectin I-B4 specifically labels subclasses of small-diameter trigeminal afferent fibres in the rat (3). GSA I-B4 labelling was most intense in the cytoplasm with high magnification. It is likely that the plasma membrane and Golgi apparatus were labelled (4).

It has been demonstrated that the peripheral processes of the trigeminal ganglion cells express GSA I-B4 within the ophthalmic and maxillary nerves and the sensory component of the mandibular nerve of the trigeminal nerve (10, 15, 16). The distribution of GSA I-B4-positive trigeminal ganglion cells has been studied previously (3, 9, 10) but the localisation of them within the ganglion is unclear. In the present study, GSA I-B4-labeled fibre bundles from the three branches of the trigeminal nerve entered to the ganglion and reached to their cell bodies located in discrete regions within the ganglion. In view of the nature of non-peptide containing afferents, which could be sensory C-fibres and may subserve nociceptive function (16, 22), the localisation of GSA I-B4-pozitive neurones within the ganglion correlates well with the data (18) in which it has been suggested that the cell bodies of sensory neurones for the three divisions of the trigeminal nerve occupy anatomically discrete regions within the ganglion.

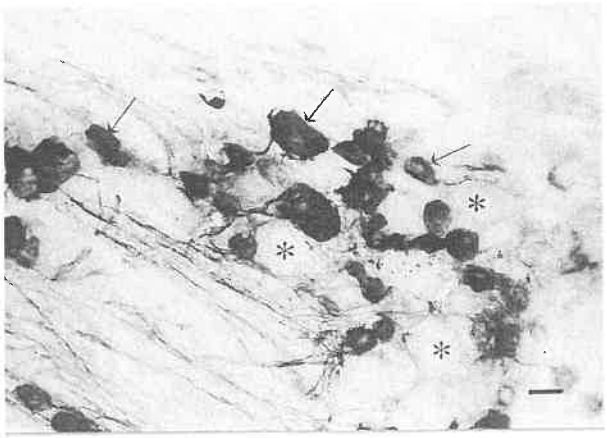

Figure 1. High magnification of small neurones stained with the GSA I-B4 lectin in a section of trigeminal ganglion (arrows). Lectin-positive cell bodies exhibit intensive cytoplasmic reactivity, while large neurones are lectin-negative (stars). Scale bar: 50 μm

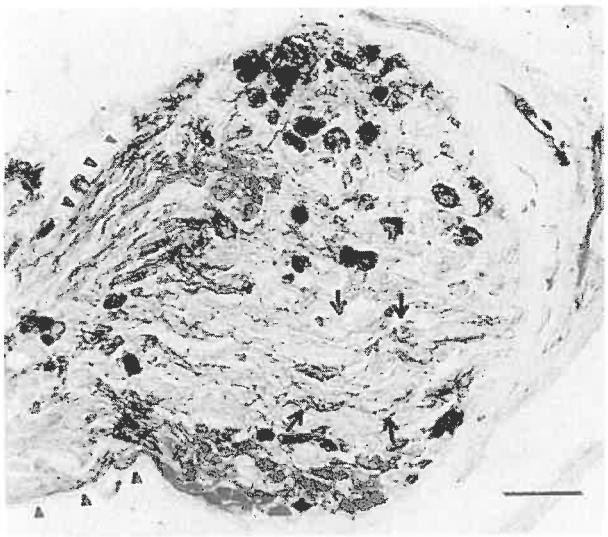

Figure 2. Low magnification of neurones labelled by GSA I-B4 in trigeminal ganglion. The vast majority of labelled neurones are seen at the poles of the ganglion. Lectin-positive axons from the branches of the

trigeminal nerve reach to their cell bodies at these regions (arrowheads). A bundle of lectin labelled fibres is seen in the middle of the ganglion (arrows). Scale bar: 100 μm

As mentioned before the cell bodies of most primary sensory neurones are in the trigeminal ganglion with the remainder being in the mesencephalic trigeminal nucleus (18). In the present study, most of GSA I-B4-positive trigeminal afferent fibres from the three branches of the trigeminal nerve terminated in the ganglia, but a bundle of labelled fibres traversed through the ganglion. These fibres could terminate at mesencephalic trigeminal nucleus (3). The localisation of peptidergic, CGRP or substance P, primary afferents in trigeminal ganglion (23-26) does not seem to co-localise with non-peptidergic afferents within the ganglion. In the present study, localisation of the non-peptidergic afferents was found in the poles of the ganglion, while the peptidergic afferents had been found through the ganglion (23). Few co-localisations have been reported between GSA I-B4-labeled cells and CGRP containing cells in trigeminal ganglion (3). Hence our data would support the use of GSA I-B4 as a marker for non-peptide primary afferents (3, 22).

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Yazışma Adresi:
Zafer Soygüder
Yüzüncü Yıl Üniversitesi
Veteriner Fakültesi
Anatomi Anabilim Dalı
VAN / TÜRKİYE