ARAŞTIRMA

F.Ü.Sağ.Bil.Vet.Derg.
2012; 26 (1): 27 - 30
http://www.fusabil.org

Immunohistochemical Localization of Calbindin-D28k in Porcupine (*Hystrix cristata*) Trigeminal Ganglion

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Histologically light microscopic structure, and immunohistochemically the presence and distribution of calbindin-D28k was investigated in the trigeminal ganglion of porcupine (*Hystrix cristata*). Many calbindin-immunoreactive neurons were distributed throughout the ganglia. All immunopositive cells were medium to large in size. Small ganglion neurons were not stained. There were three types of neurons as small, medium and large. Most of the neurons were enveloped by small satellite cells. The shapes of satellite cells were polimorphal and polygonal.

Key Words: *Calbindin-D28k,immunohistochemistry, porcupine, trigeminal ganglion.*

Oklu Kirpi (*Hystrix cristata*) Trigeminal Gangliyon' unda Calbindin-D28k'nın İmmunohistokimyasal Lokalizasyonu

Oklu kirpi (*Hystrix cristata*) trigeminal gangliyonunda histolojik olarak ışık mikroskopik yapı, immunohistokimyasal olarak ise calbindin-D28k'nın varlığı ve dağılımı araştırıldı. Çok sayıda calbindin-pozitif nöron gangliyona dağılmıştı. Pozitif hücrelerin hepsi büyük ve orta hacimliydi. Küçük gangliyon nöronları boyanmadı. Küçük, orta ve büyük hacimli olmak üzere 3 tip nöron vardı. Nöronların çoğu küçük satellit hücrelerle kuşatılmıştı. Satellit hücrelerin şekli polimorfal ve poligonaldı.

Anahtar Kelimeler: *Calbindin-D28k, immunohistokimya, oklu kirpi, trigeminal gangliyon.*

Introduction

Calcium plays an important role in the synthesis and release of the neurotransmitters and is related to metabolic activity of the cells. To act, it requires a variety of calcium-binding proteins. Calbindin-D28k, a major calcium-binding protein, is widely distributed to neurons in the nervous system, and it is generally used as a neuronal marker for neuroanatomical studies. This protein may serve as a buffer protein to regulate level of intracellular calcium (1).

Sensory neurones of the trigeminal ganglia are very important. Because the trigeminal ganglia are associated with the sensation of the face. Additionally, the sensory nerve fibers innervating the cerebral blood vessels originate in trigeminal ganglion (2). Ichikawa et al., (3) reported that the tooth pulp was innervated by nerve fibers with calbindin-D28k immunoreactivity which originated from trigeminal gaglion.

Trigeminal ganglion is composed of pseudounipolar neurons, satellite cells and their fibers. Pseudounipolar neurons in trigeminal ganglion are sensory cells. The perikarya of them are tightly wrapped by small satellite cells (4,5).

Calbindin-D28k immunoreactivity in the trigeminal ganglion has been extensively documented in some species such as rat (6, 7), goat (8), cat (9), guinea pig (10) and mouse (11). However, the existence of calbindin-D28k immunoreactivity has not been reported in the trigeminal ganglion of porcupine.

Therefore, the aim of the present study was to identify calbindin-D28k-containing neurons in the trigeminal ganglion of the porcupine, to provide more detailed information about their distributional patterns.

Materials and Methods

Four (four pairs of trigeminal ganglion) adult porcupines (*Hystrix cristata*) were used in this study. They were caugth by villagers in Eastern Anatolia (Turkey). Deep anaesthesia of animals was induced by initial injection of (1.5 cc *i.m.*) cetanes (ketamine HCL) followed by (3.0 cc *i.m.*) rompun (xylazine HCL). The experimental study was carried out in accordance with ethical considerations. Small pieces of tissues were dissected from trigeminal ganglion was removed immediately and placed in 10% formalin in phosphate-buffered saline (PBS), pH 7,4, for 18 hour before paraffin embedding. Tissue samples were routinely processed through a graded series of

Geliş Tarihi : 26.12.2011
Kabul Tarihi : 10.01.2012

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alcohols, cleared in xylol and embedded in paraffin. 5 μm thick sections were obtained and processed for immunohistochemical staining.

Immunohistochemical staining was carried out by using the streptavidin-biotin complex technique (12). Blocking of endogenous peroxidase activity was carried out with 0.08% hydrogen peroxide (H_2O_2) in methanol for 5 minutes. In order the block non-specific binding, an incubation with Large Volume Ultra V Blok (Lab Vision) for 30 min. was performed.

Sections were incubated for 16-20 hours at 4 °C in mouse anti-calbindin (Sigma). Antibody was diluted to 1:200 with PBS containing 0.25% sodium azide and 2.5% bovine serum albumin respectively. Sections were then incubated in biotinlated secondary antiserum (Lab Vision) for 1 hour, followed by rabbit streptavidin-biotin - peroxidase complex (Lab Vision) for 1 hour, at room temperature. Some of sections were washed in PBS for 30 minutes after each incubation and finally immersed in AEC (Dako) chromogen substrate for 10 minutes. After washing in distilled water, the sections were counterstained with Mayer Hematoxylin. The sections were dehydrated and coverslips mounted with squamos mounting medium. The other sections were visualized using glucose oxidase- diamino benzidine (DAB), nickel ammonium sulphate (GDN) substrate (13) for 10 minutes. After washing with distilled water, the sections were counterstained with hematoxylin. The sections were examined with light microscope and photographs were taken (Olympus 13X51, JAPAN).

Results

Immunohistochemical Observations: We observed calbindin-D28k immunoreactive neurons in the trigeminal ganglia. A large number of neurons exhibited calbindin-D28k immunostaining. Immunoreactivity was observed in two neuronal forms including medium and large cells and, evenly distributed in both medium and large cells (Figure 1, 2). The medium-sized neurons comprised the largest calbindin-positive population. The most intensely stained cells were medium ones, the large sized ones were moderately stained. The intensity of staining varied from light- to dark-blue. Immunoreactivity homogeneously distributed within the cytoplasm. The labeled neurons were scattered throughout the ganglia, but was not uniform. They showed no regional preference in porcupine (Figure 2). Pericellular basket-like formations surrounded most of the ganglion neurons with calbindin-D28k immunoreactivity were observed (Figure 1, 3). However, small ganglion neurons were not markedly stained (Figure 2, 3).

Light Microscopic Observations: On histological sections, there were three types of neurons according to their size in porcupine trigeminal ganglia: small, medium and large (Figure 4). The large neurons had a light cytoplasm. The medium and small sized neurons had the largest population of neurons situated in trigeminal ganglion. The small neurons were round to oval in appearance. Their nucleus was situated in the centre of the cytoplasm. The small and medium-sized neurons were seen light and dark according to the color of their cytoplasm. The medium neurons generally were with polygonal shape. It was visualized that the perikarya of the pseudounipolar cells were enveloped by small satellite cells. The shapes of satellite cells were polimorphal and polygonal. Their outer surface was covered with basal membrane, separated satellite complex from endoneurinum. But, there was no basal membrane in the some places of tight contact of two satellite cells. Their number was different and generally depended on the size of the neuronal cell. They usually were positioned on certain distances, and sometimes they formed a ring around cell (Figure 3, 4).

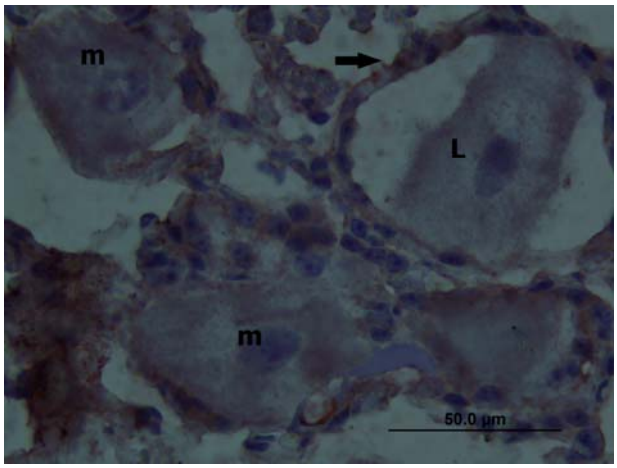

Figure 1. Medium (m) and large-sized cells (L) were calbindin-positive. Pericellular basket-like formations with calbindin-D28k immunoreactivity were observed (arrow). AEC method.

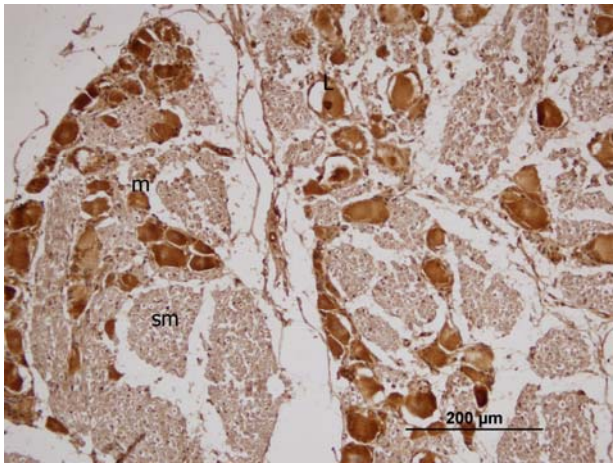

Figure 2. Section from the trigeminal ganglion demonstrating a dense accumulation of large (L) and medium-sized (m) calbindin-D28k positive cells. However the small neurons (sm) were negative. DAP method.

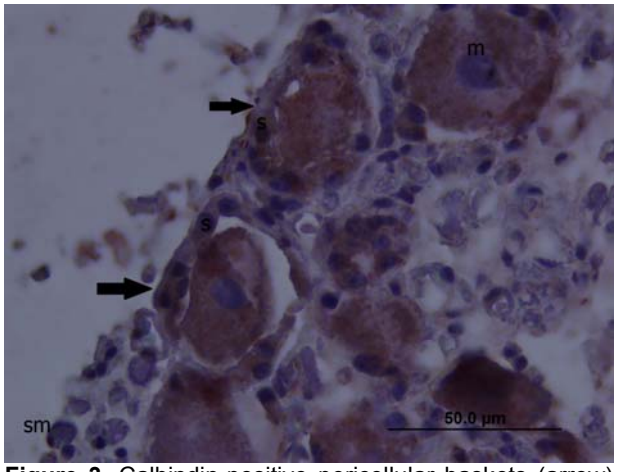

Figure 3. Calbindin-positive pericellular baskets (arrow) surrounded the most of ganglion neurons in trigeminal ganglion. Perikarya of neurons were enveloped by small satellite cells (s). Calbindin-positive medium-sized cells (m) and calbindin-negative small cells (sm) presented. AEC method.

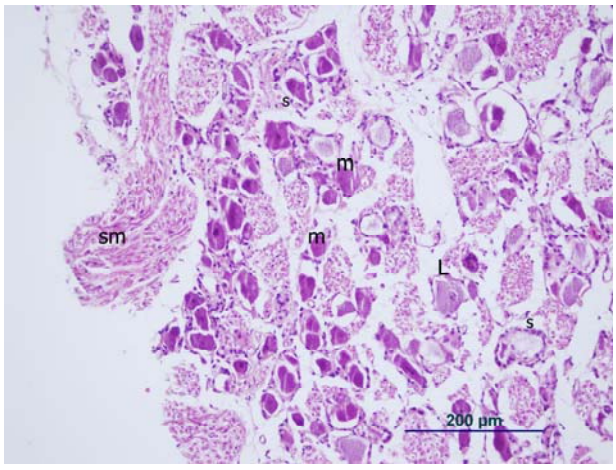

Figure 4. Neurons with different size was observed in trigeminal ganglion. L: large-sized cells m: medium-sized cells, sm: small-sized cells. Satellite cells (s) disposed around the perikarya of neurons. H.E.

Discussion

In the present study, we observed neurons with calbindin-D28k immunoreactivity in the trigeminal ganglion of porcupine.

Neurons encircled by nerve fibers are called 'pericellular basket-like formations' (14). Immuno-positive pericellular baskets have been reported in the guinea pig trigeminal ganglion (10, 15, 16). However, Shimizu et al. (6) couldn't detect any pericellular basket-like formations in the rat. In our study, pericellular basket-like formations of ganglion neurons were observed. This study shows the possibility that species difference may affect the occurrence of pericellular basket-like formations in neurons of trigeminal ganglion.

Shin et al. (8) reported that calbindin-D28k immunoreactivity was observed in small and large cell forms and immunoreactivity was evenly distributed in both small and large cells. Lazarov (9) and Ichikawa et al. (11) suggested that calcium-binding proteins were mainly localized at large neurons. Shimizu et al. (6) described large, medium and small-sized calbindin-immunoreactive neurons. According to Shimizu et al. (6) small- sized neurons were the most common. In the present study, immunoreactivity was observed in two neuronal forms including medium and large cells and, evenly distributed in both medium and large cells.

Beaver et al. (17) described lack of basal membrane in some parts of tight contact of two satellite cells. This report is in aggreement with our results.

Ichikawa et al. (11) suggested that calbindin-immunoreactive neurons showed no topographic preference in mouse. Similarly, our results showed that immunoreactive calbindin-D28k neurons were widely distributed to all region of the trigeminal ganglion.

Shin et al. (8) reported that almost all neurons were round-shaped in trigeminal ganglion of goat. In present study, the neurons were in different shapes.

In general our study is in agreement with the results of many authors working on trigeminal ganglion of different animals. However, there were some spesific features in porcupine trigeminal ganglion.

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