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DISTRIBUTION OF THE CALBINDIN D LABELLING IN THE RAT SPINAL CORD

Ratların Omuriliğinde Calbindin D'nin Dağılımı

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SUMMARY

The localization of Calbindin D in the lumbar spinal cord was investigated in the rat using immunocytochemistry. Considerable regional variations were observed. The antibody to Calbindin D resulted in a dense staining in lamina I and II light staining in rest of the laminae. Occasional scattered cells were seen in the deep laminae and few in the lamina X, ventral horn and in the lateral spinal nucleus.

These results demonstrated that Calbindin D expressing neurons are separate populations of small Islet cells in the dorsal horn of the spinal cord. Our findings suggest that these neurons are probably excitatory interneurons.

Key Words: Rat, Spinal cord, Calbindin D.

ÖZET

Ratlarda lumbal omurilikte immunositokimyasal metot yoluyla Calbindin D'nin lokalizasyonu araştırıldı. Calbindin D'in lumbal omurilikteki localizasyonu bakımından belirgin bölgesel farklılıklar gözlendi. Calbindin D antibodi'sinin immunositokimyasal dağılımı lamina I ve II de koyu, diğer laminalarda ise açık bir boyama ile sonuçlandı. Laminaların derin kısımlarında seyrek olarak dağılmış, lamina X, ventral kornu ve lateral spinal nukleus'da birkaç adet olmak üzere hücreler gözlendi. Bu sonuçlar omuriliğin dorsal kornularında, Calbindin D'yi exprese eden sinir hücreleri, küçük islet hücrelerinin farklı bir populasyonu olduğunu ortaya çıkarmıştır. Bulgular, sinir hücrelerinin muhtemelen eksitatör internöronlar olduğunu göstermiştir.

Anahtar Sözcükler: Rat, Omurilik, Calbindin D.

INTRODUCTION

Previous studies have shown that calbindin-D28k, calmodulin, parvalbumin and calretinin which are members of the "EF-hand" family of calcium-binding proteins (1), can be used as markers of specific subpopulations of neurons in the dorsal harn of the spinal cord (2,3). As these protein are distributed, throughout the cytoplasm of neurons that contain them, immuunocytochemistry can result in extensive staining of the dendritic trees, and thus allow the morphology of immunoreactive neurons to be examined. Calcium-binding proteins have been identified, both in the adult spinal cord (4-7) and various regions of the brain (3,5,8,9). Parvalbumin-IR (immunoreactive) neurons are scattered in LII and III of the dorsal horn, while calbindin D-IR neurons occur in LI-III. It has been shown that most parvalbumin-IR neurons in LII and LIII were GABA-IR, whereas very few calbindin D-IR neurons were GABA-IR (2).

MATERIALS and METHODS

Eight adult wistar rats, weighing approximately 250g of either of sex were deeply anaesthetized and perfûsed with a fixative containing 4% paraformaldehyde in 0.1M phosphate buffer saline (PBS). Lumbar spinal cord segments were removed, postfixed in fixative (4-6 h) and then cryoprotected with 30% sucrose in 0.1 M PBS overnight at 4 °C. Tissue blocks were cut on a freeze knife microtome into 40 μm transverse sections and processed free-floating.

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The endogenous peroxides and non specific binding sites for antibodies were suppressed by treating sections with 1% hydrogen peroxide for 30 minutes and 10% normal donkey serum for an hour at room temperature respectively. Further, sections were processed for standard immunocytochemistry by the avidin-biotin-peroxidase (ABC) method (10).

The sections were incubated in primary antiserum to calbindin D (Sigma) diluted 1:200, in PBS containing, bovine serum albumin (BSA) (2.5%) and TritonX-100 (2%), overnight at 4 ºC. Subsequently the binding of primary antisera was detected using biotinylated anti-mouse antisera (1:300) and streptavidin-conjugated horseradish peroxidase (1:1000) (both from Amersham). Finally the chromogen protocol of Shu et al (11) used to reveal the distribution of bound peroxidase.

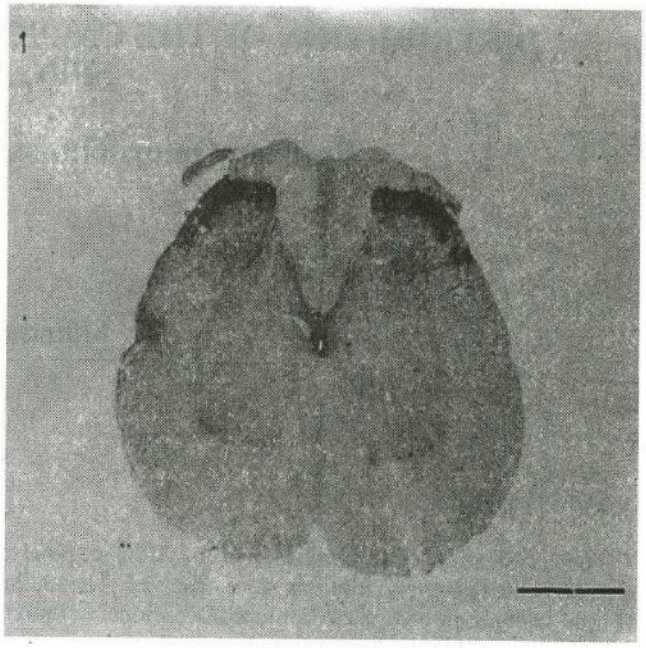

Figure 1. Low power of photomicrograph shows pattern of the calbindin D distribution in the lumbar spinal cord.
Resim 1. Lumbal omurilikte calbindin D'nin dağılımı (x2.5).

RESULTS

Calbindin D immunoreactivity in LI and II was seen as dark punctate staining with a few round cells on the border of LI and large numbers of small neurons in LII, some of which gave rise to stained dendrites or process (Fig. 1,2,3). Calbindin D-IR neurons (soma diameter; 7$_{\mu}$m, mean intersoma distance; 15$_{\mu}$m) had one or two short dendrites which could be followed for 2-6$_{\mu}$m. They tended to have a dorsally to ventrally orientation and occasionally medially to laterally. Calbindin D-IR cells were seen mostly in LII and some in LIII (Fig. 3), occasionally a few cells were seen in LV (Fig. 4) and in lateral spinal nucleus (LSN) (Fig. 6)-B). LV cells (soma diameter; 7.5 $_{\mu}$m) had 3-4 dendrites which ran all directions (Fig. 4). In LX (Fig. 5) and occasionally in the ventral horn neurons were observed which showed nuclear staining and weak cytoplasmic staining. In LSN, calbindin D-IR cells (soma diameter; 11$_{\mu}$m) had 2-4 dendrites (mean dendrites length; 16$_{\mu}$m) extended medially and laterally (Fig. 6).

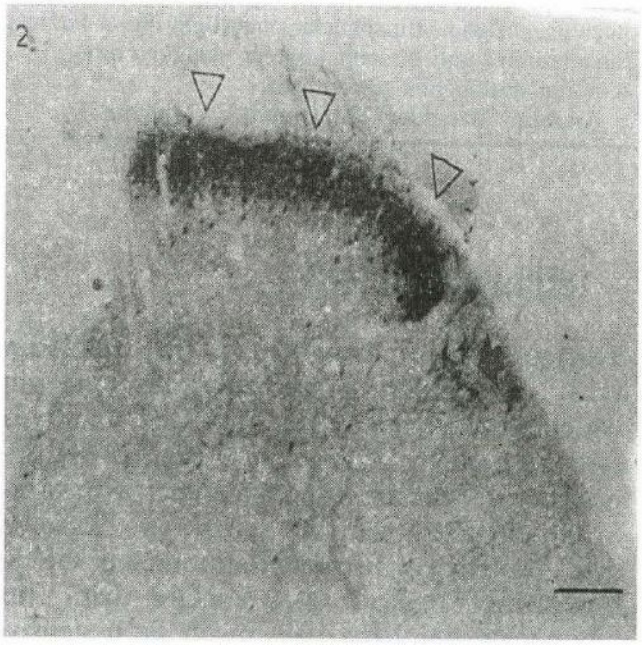

Figure 2. Low power view of photomicrograph to illustrate the pattern of the calbindin D staining in the dorsal horn (arrowheads) and LNS (arrows).
Resim 2. Dorsal kornu ve lateral spinal nukleusta calbindin D'nin dağılımı (x10).

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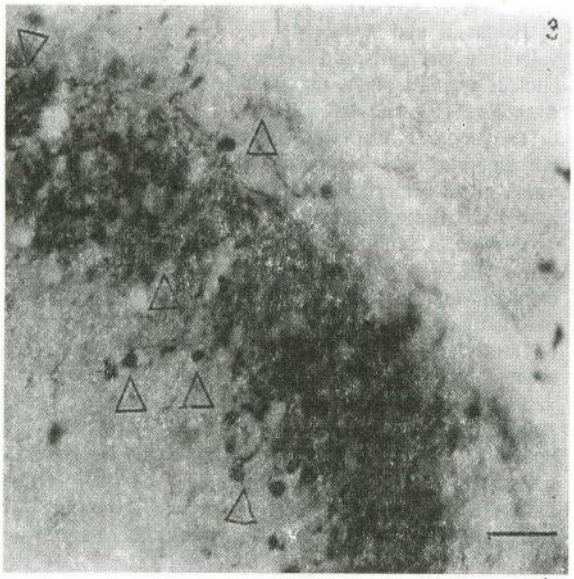

Figure 3. High power view of photomicrograph to illustrate the pattern of the calbindin D staining in the dorsal horn. Heavily labelled neurons and small process were concentrated in LI and particularly LII of the lumbar spinal dorsal horn (arrows)
Resim 3. Dorsal kornu da calbidin D'nin dağılımı (x40). Lumbal omuriliğin dorsal kornu'sunda koyu olarak boyanmış nöronlar ve küçük uzantıları LI ve özellikle LII de yoğunlaşmaştır.

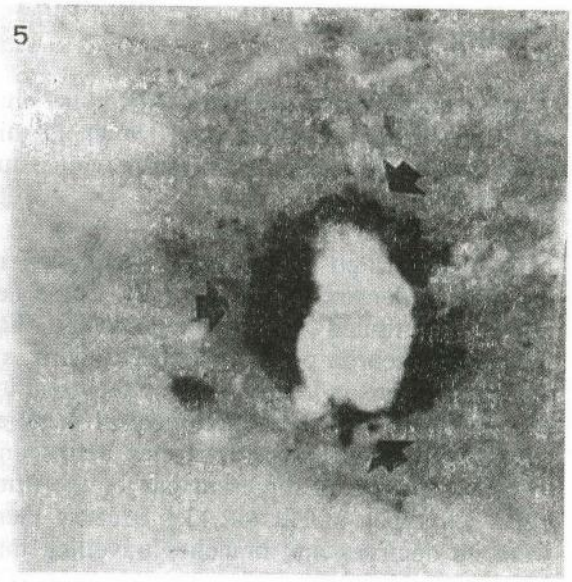

Figure 5. Calbindin D-IR is in the LX. Heavily stained ependymal cells are seen in the lining of the central canal (arrows).
Resim 5. LX'de calbindin D'nin dağılımı. Canalis sentralis etrafını saran ependymal hücrelerinin görüntüsü.

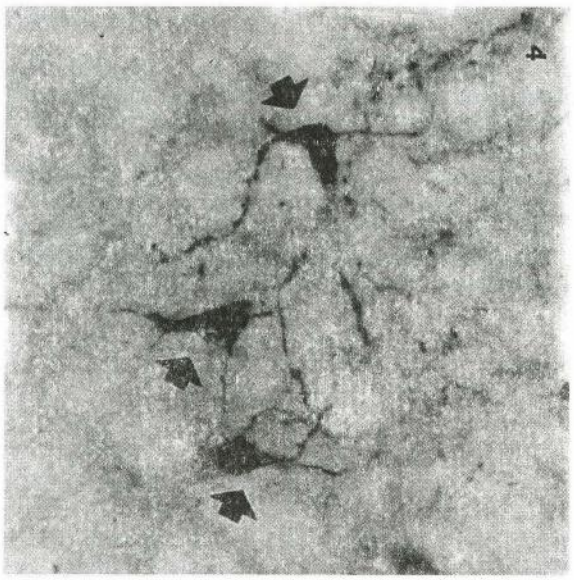

Figure 4. A few calbindin D cells are in the neck of the dorsal horn (LV) (arrows)
Resim 4. Dorsal kornu'nun boyun kısmında bulunan calbindin D hücreleri.

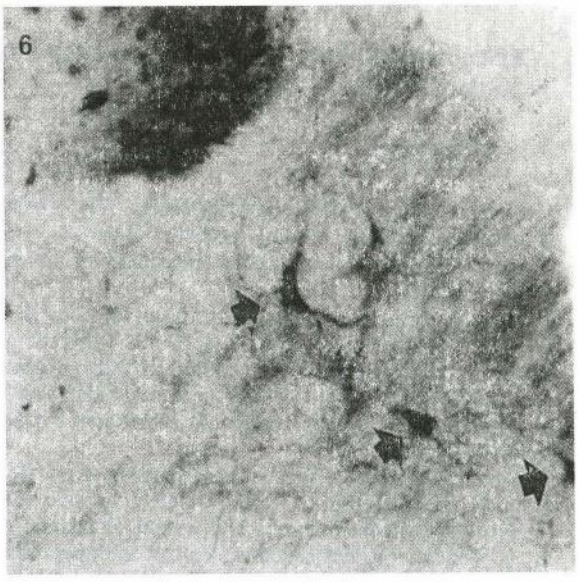

Figure 6. A few calbindin D cells are in the LSN (arrows)
Resim 6. Lateral spinal nukleus'daki calbindin D hücrelerinin görüntüsü.
Scale bars: 1; 500 μm, 2,3,4,5 and 6; 250 μm

DISCUSSION

The distribution of calbindin D-IR in the dorsal horn of the rat spinal cord shown in this study was in agreement with the previous studies (2,4-7).

Calbindin D positive neurons were seen in the highest density in LII and LIII. These neurons constituted a morphologically homogeneous neuron population. The majority of labelled neurons in LII presumably represent local interneurons. Theirmorphological features suggest that axons of neurons may correspond to Islet cells which represent one of the principal cell types in LII (12,13), Because there were no dentrites and branches travelling into LI in these laminae. Their dentritic processes were located in LII. LIII cell bodies and dendritic arbors also resemble the Islet cells of LII (14). Calbindin D-IR is co-localised with glutamate-like-IR in a population of LII Islet cells (15). Speculatively it marks a population of excitatory interneurons.

These observations support the view that calbindin D expressing neurons are separate populations of small Islet cells. These neurons are probably excitatory interneurons.

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