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Calbindin-D28k immunoreactivity in the pineal gland of the adult porcupine (*Hystrix cristata*)

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ABSTRACT

Distribution of the calbindin-D28k, an intracellular protein with a high affinity for calcium, was investigated immunohistochemically in the pineal gland of porcupine (*Hystrix cristata)*. Two different types of cells were calbindin-D28k positive. The first was characterized by small sized cells. The second type of calbindin-D28k positive cells were characterized by their large size. Immunoreactive cells were found throughout the whole pineal gland without any preferential location, however they presented more densely around the base of the pineal stalk. Interstitial cells were calbindin-D28k negative.

Key words: Calbindin-D28k, pineal gland, porcupine, immunohistochemistry

Introduction

The pineal gland is an important component of the photoneuroendocrine system of vertebrates (OKSCHE and HARTWIG, 1975). It contains mainly pinealocytes and glial cells, pinealocytes being the secretory elements release melatonin, which is secreted in a daily rhythmic manner with a nocturnal peak (POCHET et al., 1994; SHIMIZU et al., 2003). Calcium appears to play an important role in melatonin production (WHITE and KLEIN, 1993). Actually, the nocturnal rise in melatonin may be prevented with the addition of inorganic calcium channel blockers. Likewise, chelation of extracellular calcium blocks melatonin output (ZATZ and MULLEN, 1988).

Calbindin-D28k, a major calcium binding protein, has been expressed in many organs including the cerebellum, kidneys (TIMURKAAN et al., 2003; AYDIN et al., 2005;

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TIMURKAAN et al., 2006; KARAN et al., 2007) and retina (POCHET et al., 1991). However, it is usually used as a neuronal marker in the central nervous system.

Calbindin-D28k has been documented immunohistochemically in the pineal glands of various mammals (KRSTIC and NICOLAS, 1988; KRSTIC, 1988; BASTIANELLI and POCHET, 1994a; BASTIANELLI and POCHET, 1995). But, immunohistochemical localization of calbindin-D28k in the pineal gland of porcupine has not yet been described.

The present study was undertaken with the aim of identifying calbindin-D28k immunoreactive cells in the porcupine pineal gland.

Materials and methods

Four adult porcupines (*Hystrix cristata*) were used in this study. They were captured by villagers in Eastern Anatolia (Turkey). The experimental study was carried out in accordance with ethical considerations. Small pieces of tissues were dissected from the pineal gland after death and placed in 10% formalin in phosphate-buffered saline (PBS), pH 7.4, for 18 hours before paraffin embedding. Tissue samples were routinely processed through a graded series of alcohols, cleared in xylol and embedded in paraffin. Five μm thick sections were obtained and processed for immunohistochemical staining.

Immunohistochemical staining was carried out using the streptavidin-biotin complex technique (POLAK and VAN NOORDEN, 1997). Blocking of endogenous peroxidase activity was carried out with 0.08% hydrogen peroxide (H_2O_2) in methanol for 5 minutes. In order to block non-specific binding, an incubation with Large Volume Ultra V Blok (Lab Vision co) for 30 min. was performed.

Sections were incubated for 16-20 hours at 4 °C in mouse anti-calbindin (Sigma). Antibody was diluted to 1:500 with PBS containing 0.25% sodium azide and 2.5% bovine serum albumin respectively. Sections were then incubated in biotinlated sheep anti-mouse IgG (sigma), followed by streptavidin peroxidase complex (Dako) for 1 hour, at room temperature. Some of the 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, sections were counterstained with Mayer Hematoxylin and mounted with squamos mounting medium (Figs 2 and 3). The others sections were immersed in glucose oxidase-DAB-nickel ammonioum sulphate (GDN) substrate (SHU et al., 1988) for 10 minutes, washed in distilled water and counterstained with eosine and mounted in entellan (Figs 1 and 4). Sections were examined with light microscope and photographs were taken (Olympus 13X51, Japan).

Results

Calbindin-D28k immunoreactive cells were located along the perivascular spaces of the adult porcupine pineal gland (Fig. 1). Two different types of cells were calbindin-D28k

positive. The first was characterized by small sized cells. These cells showed strongly calbindin-D28k immunoreactivity. Their cytoplasm was darkly stained, whereas the nuclei appeared as clear unstained areas. Some cells showing calbindin-D28k positivity occurred in a cluster of more than two cells. The rest were found singly. They had a variety of shapes, with one or more processes. The processes were usually short. They were located in an array of positive fibers. Some of them contained a cytoplasmic lipid droplet that was easily distinguishable (Fig. 2). The second one, of calbindin-D28k positive cells, were characterized by their large size. These cells also showed strong labelling, but some of them had less intensive labelling. These large cells were preferentially distributed in the vicinity of vessels and assembled in a cluster of more than four cells (Fig. 3). The shape of the large cells was generally round. However, some had different shapes (Fig. 4). Their cytoplasm was abundant and they had a large oval nucleus, which showed invaginations and was situated eccentrically. The immunoreactive cells were distributed throughout the entire pineal gland, however they presented more densely around the base of the pineal stalk. Interstitial cells were calbindin-D28k negative.

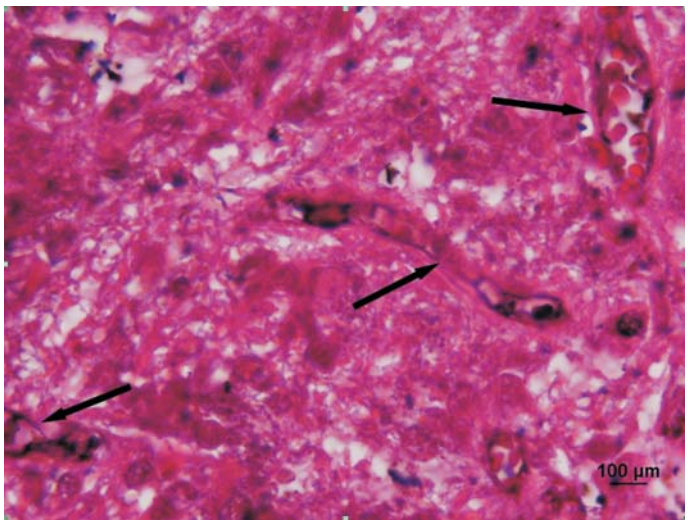

Fig. 1. Groups of calbindin-D28k-immunostained cells lining perivascular spaces in adult porcupine pineal gland (arrows)

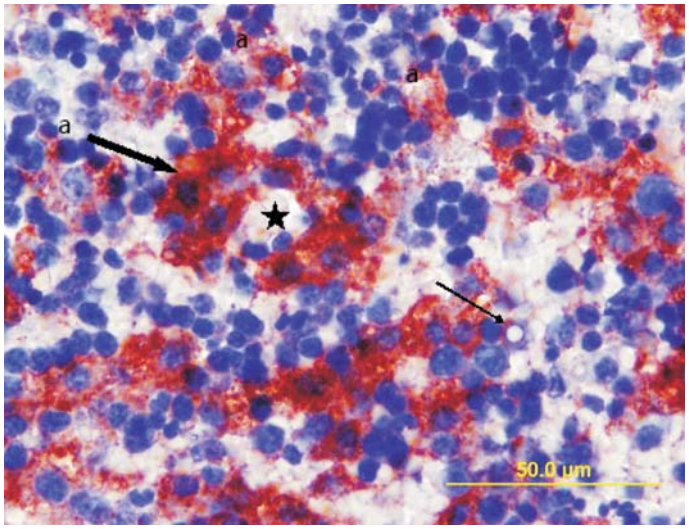

Fig. 2. Calbindin-D28k immunoreactive cells located along the perivascular space, the thick arrow indicates calbindin positive pinealocytes, the asterisk identifies capillary lumina. a: illustrates immunoreactive small cells, and the thin arrow indicates a cell presenting a lipid droplet

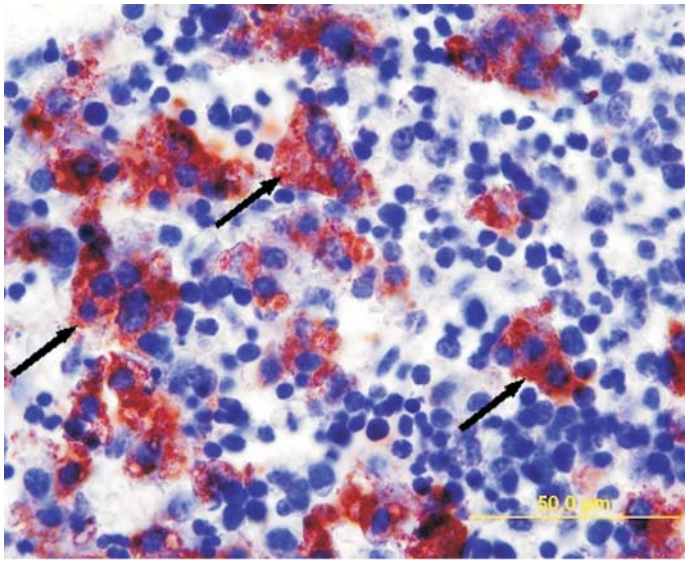

Fig. 3. The large cells assembled in a cluster of more than four cells (arrows)

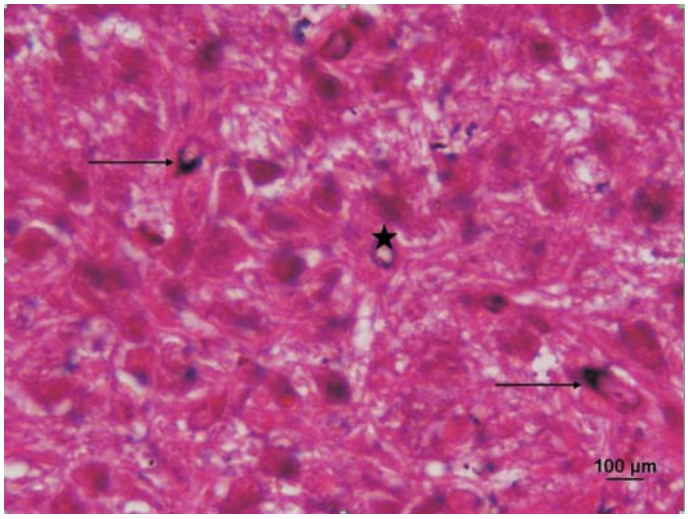

Fig. 4. Large immuno-positive cells displaying a variety of shapes (arrows) and calbindin-immunoreactive cells lining perivascular space (asterisk)

Discussion

In this study, we described calbindin-D28k immunoreactive cells in the porcupine pineal gland, and we confirmed that calbindin D28k appears as a marker of pinealocytes.

Calretinin and calbindin-D28k, two calcium-binding proteins belonging to the same superfamily, are known to act as calcium buffers. According to REDECKER et al. (1996), these two proteins are also likely to serve a calcium regulatory role in interstitial glial cells of gerbil or rat pineal glands since calretinin was expressed solely by interstitial cells, and absent from pinealocytes. The lack of calretinin and calbindin-D28k from extrapineal glial cells under physiological conditions points to the peculiar calcium-mediated functions of pineal interstitial cells.

In the rat pineal gland, calbindin-D28k is a well known marker of astrocytes (KRSTIC and NICOLAS, 1988; YAMAMOTO et al., 1990). It has been previously shown that two classes of calbindin-D28k immunoreactive cells are present according to their size in the rat pineal gland. One of the cell types is small and the other is large and perivascular. The small cell population, exclusively representing glial cells, is rather heterogenous in shape. The second class of pinealocytes is characterized by their large size (YAMAMOTO et al., 1990; BASTIANELLI and POCHET, 1993). The reports that two classes of calbindin-D28k immunoreactive cells are present and their pecularities are in accordance with our study.

BASTIANELLI and POCHET (1995) reported that large immunoreactive cells were rare in adult rat pineal glands. In our study, the calbindin-D28k immunoreactive large cells were abundant.

BASTIANELLI and POCHET (1993) reported that immunoreactive large cells were less intensively labelled in the rat pineal body. In the porcupine, the large cells generally showed a strong calbindin-D28k immunoreactivity.

POCHET et al. (1994) suggested that calbindin-D28k might not only be cytoplasma, but also nuclear and / or membrane-bound in chicken pineal glands. The nuclear localization of calbindin-D28k was observed on immunohistochemical sections by these researchers. Our work does not support the idea that calbindin-D28k immunoreactivity was localized around the nucleus.

Calbindin-D28k was detected immunocytochemically in the majority of pericapillary pinealocytes of the rat's superficial pineal body, and the interstitial cells were calbindin-D28k negative (KRSTIC and NICOLAS, 1988). In chicken, immunoreactive cells were located in the follicular and parafollicular zones (BASTIANELLI and POCHET, 1994b; POCHET et al., 1994). Our finding is similar to those of rat pineal glands.

REDECKER et al. (1996) stated that most calretinin-immunoreactive cells in gerbil and guinea pig pineal glands correspond to interstitial cells, and to pinealocytes in the Syrian hamster. In porcupine, calbindin-immunoreactive cells correspond to pinealocytes.

KRSTIC (1988) reported that there are no immunoreactive cells for calbindin-D28k in the gerbil pineal body. In view of the role of calbindin in binding and transporting calcium and regulating its intracellular levels, the absence of this protein in the gerbil pineal body has been interpreted as signifying the inability of pinealocytes to eliminate intracellular calcium, with the possible consequent formation of acervuli. In the pineal gland of porcupine calbindin-D28k immunoreactivity was present.

Calbindin-D28k in porcupine pinealocytes may be involved in the management of intracellular calcium ion (Ca^{2+}) concentrations, as observed in peripheral nerve cells by LEE et al. (1987). These results suggest that calbindin-D28k may contribute to cellular functions in the porcupine pinealocytes.

In porcupine pineal glands, the immunostaining pattern resembled the pattern which has previously been described in the rat pineal gland (KRSTIC and NICOLAS, 1988; BASTIANELLI and POCHET, 1993; BASTIANELLI and POCHET, 1995).

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KARAN, M., S. TIMURKAAN: Imunoreaktivnost moždane epifize odraslog dikobraza (*Hystrix cristata*) na kalbindin-D28k. Vet. arhiv 82, 383-390, 2012.

SAŽETAK

Raspodjela kalbindina-D28k, unutarstanične bjelančevine s velikim afinitetom za kalcij, istražena je u dikobraza (*Hystrix cristata*) imunohistokemijskim postupkom. Dva različita tipa stanica bila su pozitivna na kalbindin-D28k. Jedne su bile male, a druge velike. Imunoreaktivne stanice bile su jednakomjerno raspoređene po čitavoj moždanoj epifizi bez sklonosti nakupljanja na određenom mjestu. Ipak one su bile gušće raspoređene na bazi epifiznog stabla. Intersticijske stanice bile su negativne na kalbindin-D28k.

Ključne riječi: kalbindin-D28K, moždana epifiza, dikobraz, imunohistokemija