VETERINARSKI ARHIV 76 (3), 275-279, 2006

Distribution of Calbindin-D28k in the cerebellum of *Martes foina* - short communication

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TIMURKAAN, S., M. KARAN, Ö. ATALAR: Distribution of Calbindin-D28k in the cerebellum of *Martes foina*. Vet. arhiv 76, 275-279, 2006.

ABSTRACT

The localization of Calbindin in the cerebellum of beech marten (*Martes foina* Erxleben, 1777) has been investigated immunohistochemically using an antiserum raised against the Calbindin-D28k from chicken duodenum. Calbindin-D28k is an intracellular protein with high affinity for calcium. In the marten's cerebellum, positive staining was found in only Purkinje cells. Immunoreactive Calbindin-D28k is detected in all parts of the neuron, i.e. cell, soma, proximal processes, axons and terminals.

Key words: Calbindin-D28k, immunohistochemistry, cerebellum, *Martes foina*, stone marten, beech marten

Introduction

Calbindin is an intracellular protein that has a high affinity for Ca and vitamin-D dependent, calcium-binding proteins which has been isolated and characterized (NORMAN et al., 1982). A high molecular mass form of 28,000 Daltons (Calbindin-D28k), first identified in the intestinal epithelium of chicks by WASSERMAN and TAYLOR (1966, 1971), has also been found in various tissues in mammals, including brain and kidney (NORMAN et al., 1982; DELORME et al., 1983; STAUN et al., 1984; MARUYAMA et al., 1985), visual cortex (CELIO et al., 1986), bones, and teeth (CELIO et al., 1984).

The localization of calbindins in the cerebellum to the Purkinje cells (JANDE et al., 1981; ROTH et al., 1981; BAIMBRIDGE et al., 1982) appears uniform in all animals studied. The localization of calbindin is within the cell body, dendrites and axons of specific populations of nerve cells, as well as in the nucleus (GERMAN et al., 1997). In the nervous

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ISSN 0372-5480
Printed in Croatia

system, calbindin is primarily associated with long axon neurons, exemplified by thalamic projection neurons, cerebella Purkinje cells, large spinal, retinal, cochlear and vestibular ganglion cells (CELIO, 1990). Calbindin is believed to act as a calcium buffer, which may modulate cytosolic Ca^{+2} transients and thus protect nerve cells (HEIZMANN and BRAUN, 1992; WASSERMAN and FULMER, 1983) and as a buffer protein which prevents intracellular Ca^{+2} concentrations from reaching toxic levels during Ca^{+2} transport (ROTH et al., 1981; BRONNER and STEIN, 1988; JOHNSON and KUMAR, 1994).

The aim of present study was to examine the localization of calbindin-D28k in the cerebellum of *Martes foina* using immunohistochemical technique. Thereby, the aim was to improve knowledge of the localization and to create better understanding of the functional role of calbindin-D28k in the cerebellum.

Materials and methods

Five mabeech or stone martens (*Martes foina* Erxleben, 1777) were examined. The animals were anaesthetized and killed using ether. The cerebellum was removed immediately and placed in 10% formalin in phosphate-buffered saline (PBS), pH 7.4 for 18 hours before paraffin embedding. Tissues were routinely processed through a graded series of alcohols, cleared in xylol and embedded in paraffin. 5 μm-thick sections were obtained and processed for immunohistochemical staining.

Immunohistochemistry. Immunohistochemical staining was carried out by the peroxidase linked avidin-biotin complex (ABC) method. Blocking of endogenous peroxidase activity was carried out with 0.08% hydrogen peroxidase (H_2O_2) in methanol for 5 minutes (STERNBERGER, 1986). In order to the block unspecific binding, incubation with (1:10) normal goat serum in 0.1 M PBS, pH 7.2 was performed.

ABC Technique. Sections were incubated for 16-20 h at 4 °C in mouse anti-calbindin IgG (Sigma). The antibody was diluted to 1:500 in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin. Sections were then incubated in biotinylated sheep anti-mouse IgG (Sigma) and to follow, with streptavidin horseradish peroxidase (Dako), both at a dilution of 1:50 in PBS, for 1 h at room temperature. Sections were washed in PBS for 30 min after each incubation. Sections were then immersed in glucose oxidase-DAB-nickel ammonium sulphate (GDN) substrate (SHU et al., 1988) for 10 minutes, washed in distilled water and counterstained with eosine. Sections were examined with light microscope and photomicrographs were taken.

The specificity of each immunohistochemical reaction was determined as recommended by STERNBERGER (1979), including the replacement of specific antiserum pre-incubated with its corresponding antigen.

Results and discussion

In the beech marten cerebellum, positive staining for Calbindin-D28k was observed in the Purkinje cells only, which are located at the junction between the molecular (m) and

granular (g) layers of the cerebellum (Figs. 1 and 2). Immunoreactive Calbindin-D28k was detected in all parts of the neuron, i.e. cell soma, proximal processes, axons, and terminals (Fig. 2). Therefore, calbindin-D28k is in a position to regulate the levels of calcium throughout the neuron. The highest concentration of calbindin- D28k is in the cerebellum, where it was exclusively confined to Purkinje cell (Fig. 2) of *Martes foina*.

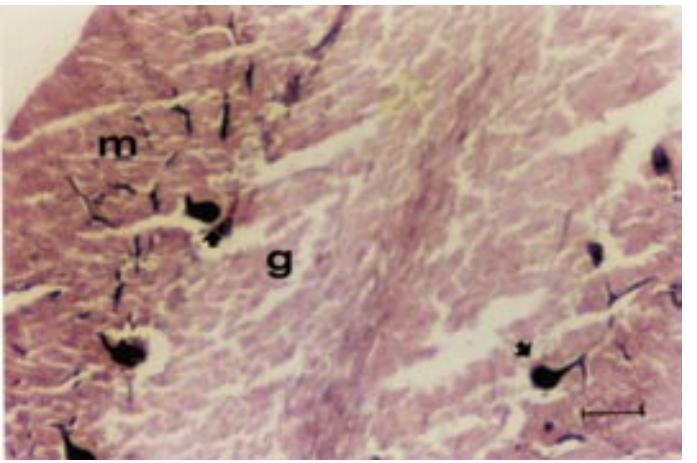

Fig.1. Calbindin-D28k immunoreactivity in the cerebellum of beech marten (*Martes foina*). Positive staining in the Purkinje cells (arrows) and their dendritic processes. Granular layer (g) and molecular layer (m). (10×5). Scale bar = 80 μm.

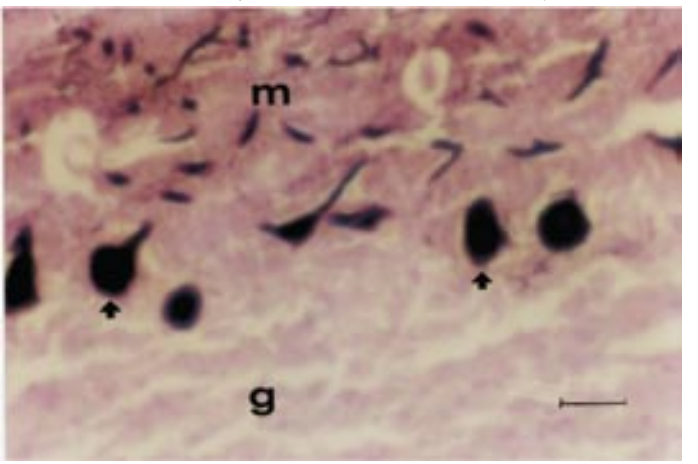

Fig. 2. Specific reactivity is seen in the Purkinje cells which are located at the junction of the granular (g) and molecular layers (m) in the cerebellum of the beech marten (*Martes foina*) (arrows). (20×5). Scale bar = 40 μm.

Results for the beech marten cerebellum are similar to those found in other rodent species and human cerebellum (JANDE et al., 1981; BAIMBRIDGE et al., 1982), where only the Purkinje cells showed a positive reaction. However, some Purkinje cells of the *Martes foina* cerebellum appear to stain more intensely than others. MUTEMA and RHOTEN (1994) have found that calbindin-D28k is localized in the cell bodies, dendrites and axons of Purkinje cells of the turtle cerebellum. We have also found the same results at the cerebellum of *Hystrix cristata* (TIMURKAAN et al., 2003)

In summary, in order to obtain a better understanding of the functional role of calbindin-D28k, we then investigated their immunocytochemical localization in the cerebellum of *Martes foina*. Immunocytochemical localization of calbindin-D28k in the cerebellum of the beech marten was described for the first time in the present study. This study showed a similarity to that of rat, rabbit and Hystrix cristata. Results of the present study may contribute to an extension of data in this field of science.

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Received: 13 January 2005
Accepted: 2 May 2006

TIMURKAAN, S., M. KARAN, Ö. ATALAR: Raspodjela kalbindina D28k u malom mozgu kune bjelice (*Martes foina*). Vet. arhiv 76, 275-279, 2006.

SAŽETAK

Nalaz kalbindina u malom mozgu kune bjelice (*Martes foina* Erxleben, 1777) istražen je imunohistokemijski upotrebom antiseruma proizvedenog protiv kalbindina D28k iz dvanaesnika pilića. Kalbindin D28k je intracelularni protein s velikim afinitetom za kalcij. Pozitivan rezultat bojenja ustanovljen je samo u Purkinjeovim stanicama maloga mozga kune. Imunoreaktivni kalbindin D28k otriven je u svim dijelovima neurona, tj. stanici, somi, proksimalnim izdancima, aksonima i živčanim završetcima.

Ključne riječi: kalbindin D28k, imunohistokemija, mali mozak, *Martes foina*, kuna bjelica