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An immunohistochemical study of distribution of calbindin-D28k in the kidney of *Martes foina* - short communication

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ABSTRACT

The localization of approximately 28,000 relative molecular mass with calcium-binding protein (calbindin-D28k) was investigated in the kidney of beech marten (*Martes foina* Erxleben, 1777). Positive cells for calbindin-D28k were found in the distal tubule by the immunohistochemical ABC technique. No reaction was seen in the proximal tubules, loops of Henle and glomeruli. Calbindin-D28k plays a significant role in calcium reabsorption in the distal convoluted tubule of kidney.

Key words: immunohistochemistry, calbindin-D28k, kidney, *Martes foina,* beech marten

Introduction

The vitamin D-dependent calcium-binding protein with molecular mass of approximately 28,000 daltons, Calbindin-D28k, also known as CaBP 28k, was discovered in 1966 by Wasserman and Taylor (WASSERMAN and TAYLOR, 1966). Calbindin-D28k belongs to a family of calcium-binding proteins that includes calmodulin, parvalbumin, troponin C and S100 (CHRISTAKOS et al., 1989; GROSS and KUMAR, 1990). This group of proteins contains the common calcium ion-binding motif known as EF-hand domain (KRETSINGER et al., 1982). Calbindin D28k has been reported in various species (WASSERMAN and TAYLOR, 1971; FULMER and WASSERMAN, 1975; RHOTEN et al., 1984 and 1986) and in many tissues, including kidney (HERMSDORF and BRONNER, 1975; RHOTEN and CHRISTAKOS, 1981; RHOTEN et al., 1985), bone (CHRISTAKOS and

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NORMAN, 1978), pancreas (POCHET et al., 1987), and the brain (BAIMBRIDGE et al., 1982; FELDMAN and CHRISTAKOS, 1983).

In kidney, calbindin-D28k is localized in the distal convoluted tubule, in the collecting tubule cells and in the cortical collecting duct cells. This distribution of calbindin-D28k has been described in chicken, rat, rabbit and man kidneys, with only very little variation (ROTH et al., 1982; TAYLOR et al., 1982; CHRISTAKOS et al., 1997).

Calbindin-D28k is expressed in high concentrations in the distal convoluted tubule of rat kidney (STAUN et al., 1984; GROSS and KUMAR, 1990). The exact functional role of calbindin-D28k in the transcellular calcium transport has not been established, but calbindin-D28k is assumed to facilitate transcellular Ca^{+2} diffusion by acting variously as a carrier protein, as a buffer protein or as a membrane Ca^{+2} transport activator (GROSS and KUMAR, 1990; BRONNER and STEIN, 1988; CHRISTAKOS et al., 1997) and as a buffer protein which prevents intracellular 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 distribution of calbindin-D28k in the kidney of *Martes foina* using immunohistochemical technique. Thereby, the aim was to improve knowledge of the localization and to create a better understanding of the functional role of calbindin-D28k in kidney.

Materials and methods

Four beech or stone martens (*Martes foina* Erxleben, 1777) were used, without sexual distinction, in this study. Under pentothal-induced (6 mL/kg) anaesthesia, the kidney was removed and immediately 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. 5μm-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 m (STERNBERGER, 1986). In order to 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 m after each incubation. Sections were then immersed in glucose oxidase-DAB-nickel

ammonium sulphate (GDN) substrate (SHU et al., 1988) for 10 m, 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 replacement of specific antiserum pre-incubated with its corresponding antigen.

Results and discussion

Immunoreactive cells were localized specifically to the distal convoluted tubules (DCT) examined of all *Martes foina* kidneys (Figs 1, 2 and 3). No specific reaction was seen in the proximal tubules, loops of Henle and glomeruli (Figs 1 and 2). Similar results were observed for the immunocytochemical localization of calcium binding protein (CaBP) in chick (ROTH et al., 1981) and rat kidney (RHOTEN and CHRISTAKOS, 1981; TAYLOR et al., 1982) using rabbit antiserum to chicken Vitamin D-dependent intestinal CaBP.

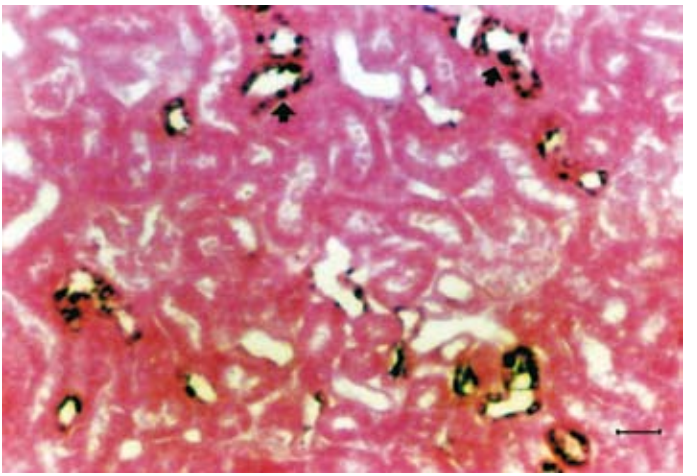

Fig. 1. Calbindin-D28k immunoreactivity in kidney of *Martes foina*. Cells positive for Calbindin-D28k are seen in the distal convoluted tubules (arrows). 10×5; scale bar = 80 μm.

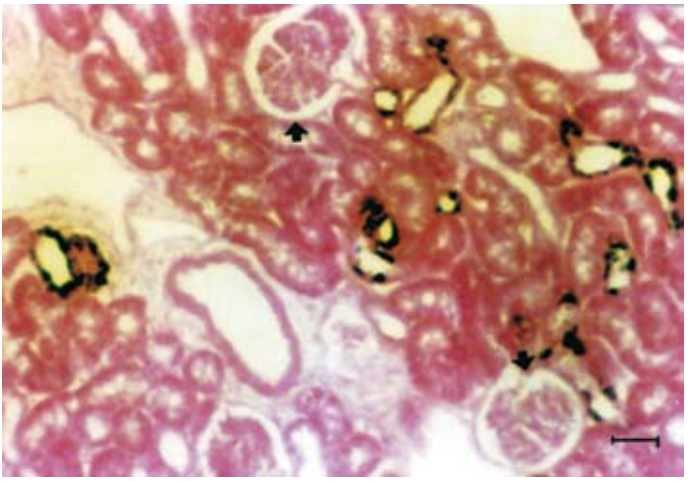

Fig. 2. Immunohistochemical localization of Calbindin-D28k in kidney of *Martes foina.* No specific reaction was seen in the glomeruli (arrows). 10×5; scale bar = 80 μm.

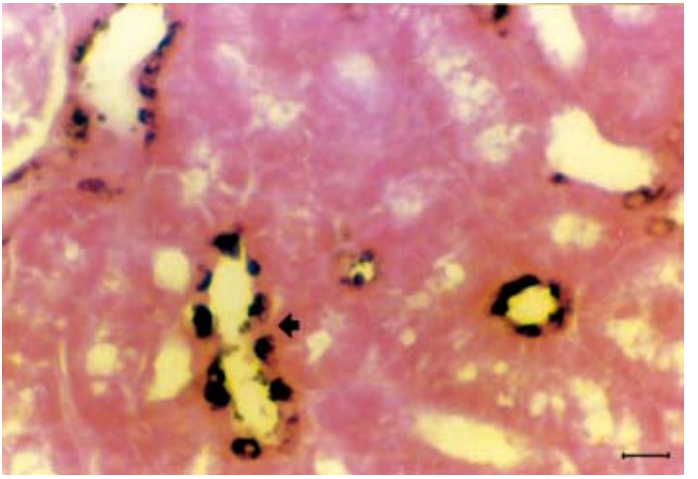

Fig. 3. Calbindin-D28k immunoreactivity in kidney of *Martes foina*. Calbindin-positive cells are seen in the distal convoluted tubules (arrow). 20×5; scale bar = 40 μm.

Calbindin-D28k was most evident in the distal convoluted tubule of kidney in amphibia (RHOTEN et al., 1986), in reptilian (RHOTEN et al., 1984), chicken (ROTH et al., 1981; CHRISTAKOS et al., 1981; TAYLOR et al., 1982), in rabbit (TAYLOR et al., 1982), in rat (TAYLOR et al., 1982; RHOTEN and CHRISTAKOS, 1981; ROTH et al., 1982), in mouse (RHOTEN et al., 1985), and in humans (ROTH et al., 1982; PARMENTIER et al., 1987).

In addition, calbindin immunoreactive sites were found to play a role in the regulation of intracellular calcium. The localization of calbindin-D28k in the distal tubule of *Martes foina* kidney indicates that calbindin-D28k may be associated with the selective reabsorption of calcium and its regulation or modulation of a number of cellular processes in kidney. These results indicate that calbindin-D28k protein has a very basic and specific role in maintaining cellular calcium homeostasis.

In summary, in order to gain a better understanding of the functional role of calbindin-D28k, we then investigated their immunocytochemical localization in the kidney of *Martes foina.* Immunocytochemical localization of calbindin-D28k in kidney of *Martes foina* was described for the first time in the present study. The result of the present study may contribute to extension of data in this field of science.

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KARAN, **M.**, **S. TIMURKAAN**, **Ö. ATALAR**: **Imunohistokemijsko istraživanje raspodjele kalbindina-D28k u bubregu kune bjelice (*Martes foina*). Vet. arhiv 77, 195-201, 2007.**

SAŽETAK

Nalaz proteina vezanog na kalcij (kalbindina-D28k) relativne molekularne mase 28000 istražen je u tkivu bubrega kune bjelice (*Martes foina* Erxleben, 1777). Stanice pozitivne na kalbindin-D28k dokazane su u distalnim tubulima imunohistokemijskim postupkom. Reakcija nije ustanovljena u proksimalnim tubulima, Henleovoj petlji i glomerulima. Kalbindin-D28k ima važnu ulogu u reapsorpciji kalcija u distalnim bubrežnim tubulima.

Ključne riječi: imunohistokemija, kalbindin-D28k, bubreg, *Martes foina*, kuna bjelica