Immunohistochemical localization of calbindin-D28k in the kidney and cerebellum of the porcupines (*Hystrix cristata*)

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ABSTRACT: The localization of calbindin in the kidney and cerebellum of *Hystrix cristata* was investigated immunohistochemically using an antiserum against the 28k Da calbindin of chicken duodenum. Calbindin-D28k is an intracellular protein with a high affinity for calcium. This protein is exclusively localized in the distal convoluted tubules of the kidney and in Purkinje cells of the cerebellum. Functionaly, calbindin-D28k is supposed to be involved in the regulation of the reabsorption of calcium in the distal nephron, though the exact regulatory mechanisms are not yet known.

Keywords: calbindin-D28k; kidney; cerebellum; *Hystrix cristata*; immunohistochemistry

Calbindin-D28k (molecular mass 28kDa) is an intracellular calcium binding protein that may be involved in trans-cellular calcium transportation and may modulate effects occurring in response to changes in intracellular calcium concentrations. It was originally purified from chicken intestine (Wasserman and Taylor, 1966). Calbindin-D28k is present in a range of various cells in all vertebrate species (Heizmann and Braun, 1995). Presence of the protein in numerous additional avian and mammalian tissues suggested an important physiological function (Christakos *et al*., 1979). The finding that it was particularly abundant in specific brain regions prompted studies investigating its particular role in calcium homeostasis (Baimbridge *et al*., 1992; Airaksinen *et al*., 1997).

There is an increasing frequency of reports describing the distribution of calbindin-D28k in brain and neural tissue: in the rat central nervous system (Garcia-Segura *et al*., 1984), and in rat brain (Shany *et al*., 1984; Pochet *et al*., 1985). The localization of calbindin is within the cell body, dendrites, axons of specific populations of nerve cells and the nucleus (German *et al*., 1997). In the nervous system, calbindin has been determined with long axon neurons, cerebellar Purkinje cells, large spinal, retinal, cochlear and vestibular ganglion cells (Celio, 1990). In the kidney, calbindin-D28k is localized in the distal convuluted tubule, in the connecting tubule cells and in the cortical collecting duct cells. This distribution of calbindin-D28k has been described in the chicken, rat, rabbit and man kidney with only very little variation (Roth *et al*.,1982; Taylor *et al*., 1982; Christakos *et al*., 1997). In addition calbindin-D28k has been detected in the distal tubules of the reptilian nephron, including saurian, ophidian, and testudine kidneys (Rhoten *et al*., 1984; Parmentier *et al*., 1987).

The exact functional role of calbindin-D28k has not yet been established (Christakos *et al*., 1997). It has been described as a carrier protein, which facilitates the trans-cellular Ca^{+2} transport (Bronner and Stein, 1988; Christakos *et al*., 1997) 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) and thus protect nerve cells (Heizman and Braun, 1992; Wasserman and Fullmer, 1983).

The rodents (*Rodentia*), which are the widest order of placental mammals, comprise more than half of the mammals known at present. Porcupine is from *Hystricidae* family, which constitutes a small group of the order *Rodentia* (Kuru, 1987; Demirsoy, 1992). However, our literature investigation showed that there was no information related to immunohisto-

chemical distribution of calbindin-D28k in tissues of *Hystrix cristata*. The objective of this investigation was, therefore, to improve the knowledge on the localization and to create better understanding in the functional role of calbindin-D28k in the kidney and cerebellum of *Hystrix cristata*.

MATERIAL AND METHODS

Adult *Hystrix cristata* was anesthetized and killed using ether. The kidney and cerebellum were removed immediately and placed in 10% formalin in phosphate-buffered saline (PBS), pH 7.4, for 18 hrs before paraffin embedding. Tissues 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 by using a 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. In order to block unspecific binding, an incubation with normal goat serum (1 : 10) in 0.1 M PBS, pH 7.2, was performed.

Sections were incubated for 16–20 hrs at 4°C in mouse anti-calbindin IgG (Sigma). The antibody was diluted to 1 : 500 with PBS containing 0.25% sodium azide and 2.5% bovine serum albumin. Then sections were then incubated in biotinylated sheep anti-mouse IgG (Sigma) followed with streptavidin horseradish peroxidase (Dako), both at a dilution of 1 : 50 in PBS, for 1 hour at room temperature. Sections were washed with PBS for 30 minutes 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 dehydrated and coverslips were mounted with mounting medium.

Sections were examined with light microscope and photomicrograps were taken.

RESULTS AND DISCUSSION

Immunoreactive calbindin-D28k was detected in all parts of the neuron cell, soma, proximal processes, axons and terminals (Figure 1 A,B). Therefore, the function of calbindin may be 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 (Figure 1 A,B arrows) of *Hystrix cristata*.

The results for the *Hystrix cristata* cerebellum are similiar 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 *Hystrix cristata* 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.

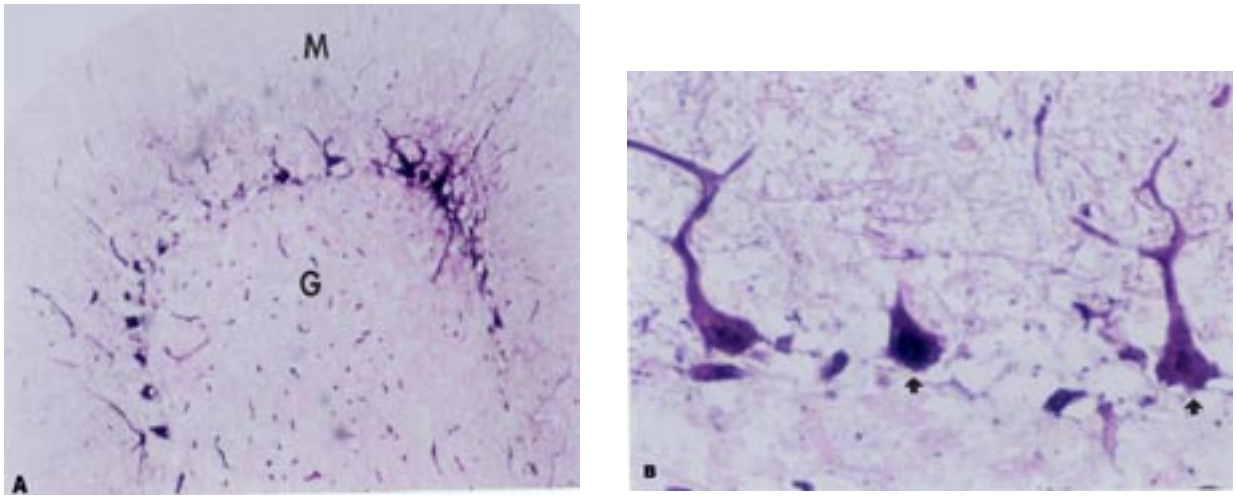

Figure 1. Immunohistochemical localization of calbindin-D28k in the cerebellum of the *Hystrix cristata*. Granular layer (G) and molecular layer (M). A – positive staining in the purkinje cells and their dendritic processes in *Hystrix cristata* cerebellum; 4 × 25. B – specific reactivity is seen in the purkinje cells which are located at the junction of the granular and molecular layers of the cerebelum (arrows); 20 × 5

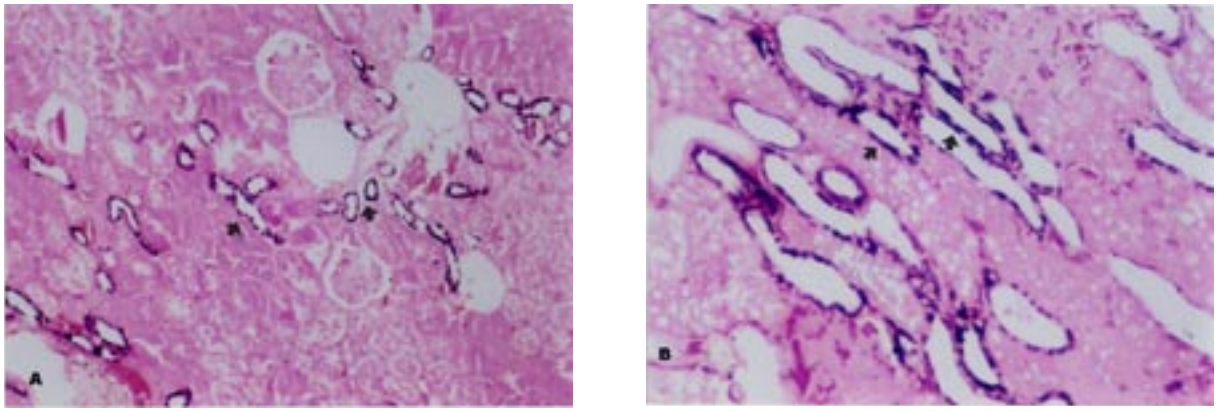

Figure 2. Immunohistochemical localization of calbindin-D28k in the kidney of the *Hystrix cristata*. A – cortical region of kidney showing positive staining in the distal convoluted tubules; 4 × 25. B – specific immunostain for calbindin-D28k (Distal convoluted tubule); 20 × 5

The role of calbindin-D28k in Purkinje cells is still open for speculation. Previous studies have suggested that this protein may have had a neuroprotective role (Iacopino and Christakos, 1990; Mattson *et al*., 1991).

In the kidney, most cells of the distal convoluted tubules in the cortical region showed a positive staining reaction with antibodies to calbindin-D28k (Figure 2 A,B). No specific reaction was seen in the proximal convoluted tubules, loops of Henle, or glomeruli (Figure 2 A). 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.

Calbindin-D28k was most evident in the distal convoluted tubule of the kidney in amphibia (Rhoten *et al*.,1986; Parmentier *et al*., 1987), in reptilian (Rhoten *et al*., 1984), chicken (Roth *et al*., 1981; Christakos *et al*., 1981; Taylor *et al*., 1982), rabbit (Taylor *et al*., 1982), rat (Taylor *et al*., 1982; Rhoten and Christakos, 1981; Roth *et al*., 1982), mouse (Rhoten *et al*., 1985), and human (Roth *et al*., 1982).

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 porcupine 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 the 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 obtain a better understanding of the functional role of calbindin-D28k, we then investigated their immunocytochemical localization in cerebellum and kidney of porcupine. Immunocytochemical localization of calbindin-D28k in the kidney and cerebellum of the porcupine was described for the first time in the present study. This study showed similarity to that observed in the rat, laboratory mouse, and other members of rodentia, or to a lesser extent to that of rabbit. The results of the present study may contribute to extension of data in this field of science.

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