

## Immunohistochemical determination of calbindin-D28k in the kidney of postnatal rats

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**ABSTRACT:** The localization of calbindin-D28k was examined in rat kidney at different stages of development. Calbindin was found specifically in the distal convoluted tubules (DCT). In the 10 day old rat kidney many cells apparently belonging to the collecting tubules were positive. In the neonatal nephrons there was also localization of calbindin-D28k to the limb of Henle, suggesting a difference in the regulation of intracellular calcium during maturation. Proximal tubule, renal corpuscle and macula densa gave no specific localization of calbindin-D28k.

**Keywords:** immunohistochemistry; calbindin-D28k; kidney; rat

Calbindin-D28k, a calcium binding protein with a molecular weight of approximately 28 000 (Christakos et al., 1989) was originally isolated from chick intestinal mucosa (Wasserman and Taylor, 1966). Calbindin-D28k belongs to a family of calcium binding proteins that includes calmodulin, parvalbumin, troponin C and S 100 (Christakos et al., 1989). This group of proteins contain 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, 1986) and in many tissues, including kidney (Hermsdorf and Bronner, 1975; Rhoten and Christakos, 1981; Rhoten et al., 1985), bone (Christakos and Norman, 1978), pancreas (Pochet et al., 1987), and the brain (Baimbridge et al., 1982; Feldman and Christakos, 1983).

In the kidney, calbindin-D28k is localized in the distal convoluted 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 human kidney with only very little variation (Roth et al., 1982; Taylor et al., 1982; Christakos et al., 1997).

The functional role of calbindin-D28k has not yet been established. It has been described as a carrier protein, which facilitates the transcellular  $\text{Ca}^{+2}$  transport (Bronner and Stein, 1988; Christakos et al., 1997), as a buffer protein which prevents intracellular  $\text{Ca}^{+2}$  concentrations from reaching toxic levels during  $\text{Ca}^{+2}$  transport (Roth et al., 1981; Bronner and Stein, 1988; Johnson and Kumar, 1994), as a modulator of insulin secretion (Sooy et al., 1999; Parkash et al., 2002) and as an inhibitor of apoptosis (Christakos et al., 2003).

Although in adult rat kidney, calbindin-D28k, has been studied in some detail, very little is known early in development. The aim of present study was to examine the distribution of calbindin-D28k in the kidney of rat different developmental stages, using immunohistochemical technique. Thereby the aim was to improve the knowledge of the localization and to foster better understanding of the functional role of calbindin-D28k in the kidney.

### MATERIAL AND METHODS

Forty Wistar rats of both sexes were examined at different developmental stages: newborn and 10,

20, 30, 40, 50, 60 day old and adult rats. The animals were anesthetized and killed using ether. The kidney was removed immediately and placed in 10% formalin in phosphate-buffered saline (PBS), pH 7.4, for 18 h 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 done with 0.08% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in methanol for 5 minutes (Sternberger, 1986). In order to block un-specific binding an incubation with (1 : 10) normal goat serum in 0.1 M PBS, pH 7.2 was performed.

**ABC Technique.** Sections were incubated for 16 to 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 followed 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 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 examined with a 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

Immunoreactive cells were localized specifically to the distal convoluted tubules (DCT) examined of all rat kidneys (Figure 1). The calbindin positive cells of the DCT did not appear to have any particular regional localization within the cortical labyrinth. In the adult and young nephrons, no immunoreactivity was seen in the proximal convoluted tubules, Henle's loops or glomeruli.

In the young nephrons, many cells apparently belonging to the wall of collecting tubules showed positive reaction. Positively stained cells of the collecting tubules were seen to extend into the medullary rays and deep into the papillae (Figure 2).

In the neonatal nephrons, there was also specific and significant localization of calbindin in the Henle's loops (Figure 3).

## DISCUSSION

In this study using the ABC immunocytochemical technique, we have been able to localize calbindin-D28k in the adult, young and neonatal rat kidney specifically to the cells of the distal tubules in which the selective reabsorption of calcium is known to occur. This localization of calbindin-D28k is similar to results obtained in kidneys of several diverse

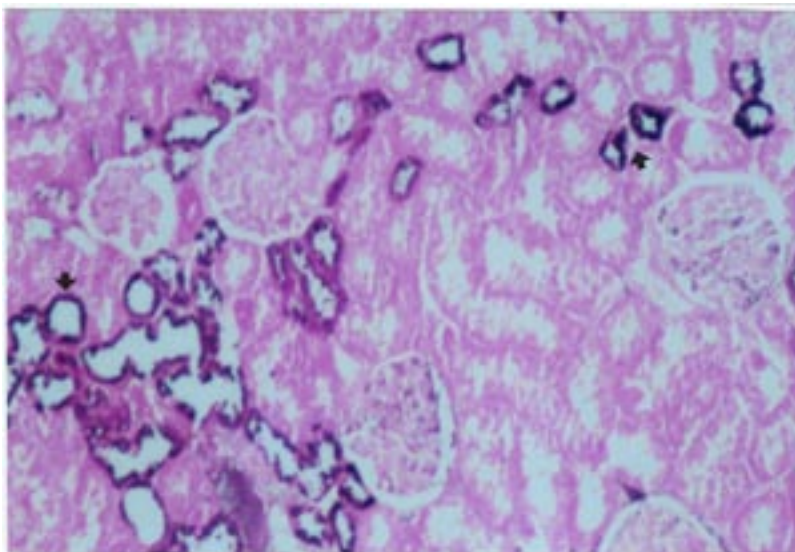


Figure 1. Calbindin-D28k immunoreactivity in adult rat kidney. Cells positive for Calbindin D-28k are seen in the distal convoluted tubules (arrows). Original magnification ×200

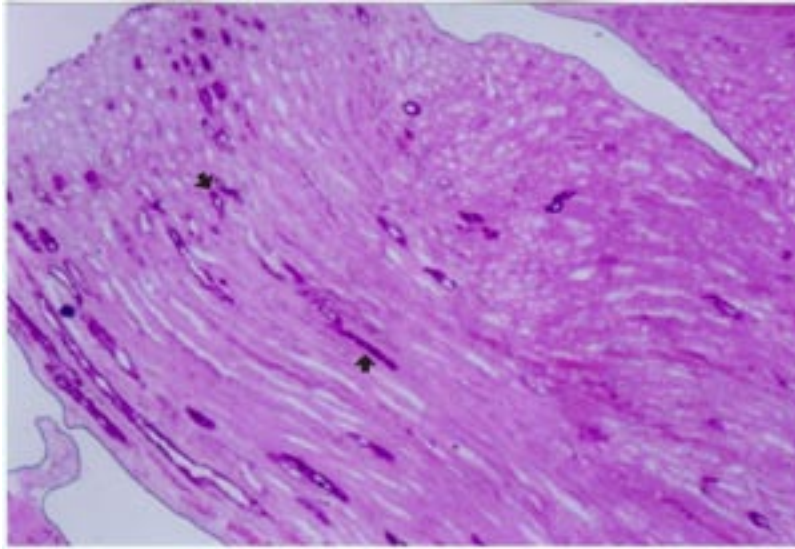


Figure 2. Calbindin-D28k immunoreactivity in the 10 day old rat kidney. Immunoreactivity was seen in the collecting tubules along the medullary rays and renal papilla (arrows). Original magnification  $\times 50$

species of vertebrates, mammalian (Rhoten and Christakos, 1981; Rhoten et al., 1985; Parmentier et al., 1987), and avian and reptilian (Rhoten et al., 1985; Parmentier et al., 1987).

In the young nephrons, not only are the collecting tubules of the cortex stained, but the collecting tubules extending along the medullary rays and deep into the papillae show a positive reaction. These results represent a more extensive distribution of calbindin-D28k than that found in the kidneys of any other animals investigated previously (Rhoten and Christakos, 1981; Taylor et al., 1982; Schreiner et al., 1983; Rhoten et al., 1984) including the human (Roth et al., 1982). This statement is not strictly true as Rhoten et al. (1982) reported immunoreactive calbindin-D28k in the medulla, including the ascending

thick limb of the loop of Henle, as well as cortical labyrinth in neonatal rat kidney. These authors suggested a unique role for calbindin-D28k during development which agrees with the suggestion of a different handling of calcium ion in the present study. This may also be suggestive of a different handling of  $\text{Ca}^{+2}$  in these animals (Schreiner et al., 1983). However there is no physiological evidence to support these observations. Since the bulk of  $\text{Ca}^{+2}$  reabsorption occurs in the proximal tubules and the ascending Henle's loops (Roth et al., 1982), this suggests that the renal calbindin-D28k does not play a role in the initial transmembrane transport of  $\text{Ca}^{+2}$ , but that it may be involved in active  $\text{Ca}^{+2}$  transport in the distal nephron and in those processes regulating intracellular  $\text{Ca}^{+2}$  levels. The localization of

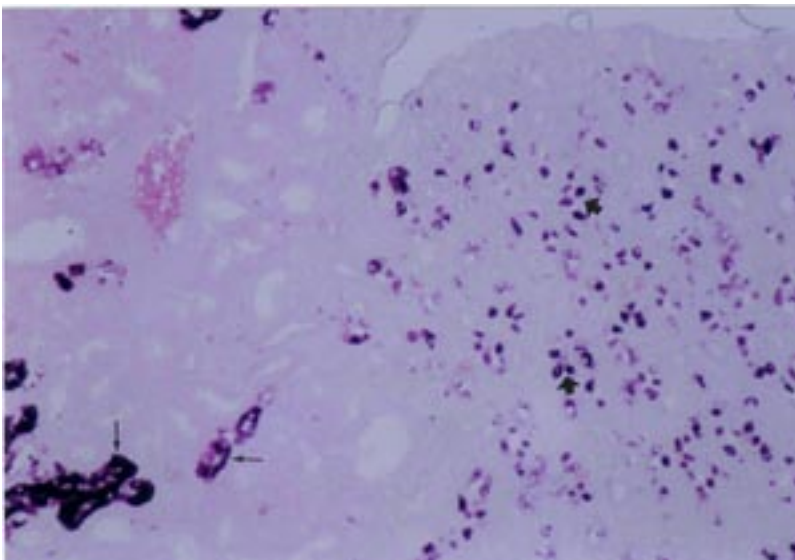


Figure 3. Calbindin-D28k immunoreactivity in the neonatal rat kidney. Immunoreactive cells were seen in the distal, convoluted tubules (thin arrows) and in the Henle's loop (thick arrows). Original magnification  $\times 50$

calbindin-D28k to a select population of cells supports the idea of an essential but highly specialized function for this protein in the kidney (Rhoten et al., 1986).

In the neonatal nephrons, there was also localization of calbindin-D28k to the limb of Henle, suggesting a difference in the regulation of intracellular calcium during maturation.

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