Localization of the thiazide sensitive Na-Cl cotransporter, rTSC1, in the rat kidney.

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Abstract

A thiazide sensitive Na-Cl cotransporter, rTSC1, has recently been cloned from a rat kidney cortex cDNA library. The molecular regulation and nephron localization of this protein is unknown. The purpose of this study was to examine the nephron distribution and subcellular localization of the rTSC1 protein in the rat kidney. In situ hybridization showed rTSC1 transcripts were localized to short, convoluted tubule segments in the kidney cortex. Polyclonal antibodies raised against a 110 amino acid segment from the amino terminus of rTSC1 recognized three major bands of 135, 140 and 155 kDa on Western blotting of membrane protein from cortex but not outer medulla of the rat kidney. Immunofluorescence studies using the antibody alone and in double labeling experiments with antibodies against the H+ ATPase and calbindin D28, showed intense labeling of apical membranes in the distal nephron beginning in the initial distal convoluted tubule and terminating within the connecting tubule. The intensity of labeling diminished from proximal to distal sites along the distal tubule. Ultrastructural studies by immunoelectron microscopy showed the cotransporter protein to be localized predominately on apical microvilli of the distal convoluted tubule cells. These results are consistent with rTSC1 encoding the apical thiazide sensitive Na-Cl cotransporter in the distal tubule.