Supplemental data

Material and methods

Plasmid construction and transfection

The full length cDNAs encoding the four subunits of wild-type (wt) human α-, β-, γENaC, and the short isoform of human δ-ENaC were used as previously described (Haerteis et al, 2009). HEK293 cells were cultivated in Modified Eagle’s Medium supplemented with 10% fetal bovine serum, 1% sodium pyruvate, 1% non-essential amino acids (all components obtained from Biochrom, Berlin, Germany) and 1% Penicillin/Streptomycin (Sigma, Taufkirchen, Germany). One day prior to transfection, cells were seeded to 60% confluence. Cells were transfected with single or all three subunits together using ExGen 500 (Fermentas, St. Leon-Rot, Germany) according to the manufacturer’s protocol.

Tissues

Normal human kidney specimens were obtained from non-affected areas during nephrectomy of renal tumors (Department of Urology, University of Erlangen-Nürnberg). All patients gave informed written consent and the study was approved by the local Ethics Committee.

Antibodies

The β- and γENaC antibodies (Pineda Antibody Service, Berlin, Germany) were used in a dilution 1:5,000 for western blotting. For immunohistochemistry of kidney tissues, the βENaC antibody was used in a dilution of 1:8,000 and γ-ENaC antibody in a dilution 1:2,000. Rat monoclonal anti-HA antibody was obtained from Roche Diagnostics (Mannheim, Germany). Sheep polyclonal anti-11-β-hydroxysteroid-dehydrogenase type II (11-β-HSD-2) was obtained from Chemicon (Schwalbach, Germany) and rabbit polyclonal anti-aquaporin 2 (AQP2) from Acris Antibodies (Herford, Germany).
Horseradish peroxidase coupled goat anti-rabbit immunoglobulin G (Santa Cruz Biotech, Heidelberg, Germany) and anti-rat immunoglobulin G (Sigma) secondary antibodies were used for Western Blotting. DyLight-conjugated goat anti-rabbit immunoglobulin G (Pierce, Rockford, Illinois, USA), FITC-conjugated donkey anti-goat immunoglobulin G and FITC-conjugated donkey anti-sheep immunoglobulin G (Dianova) were used for immunohistochemistry.

**Western blot experiments**

24 hours post transfection HEK293 cells were washed carefully in ice-cold phosphate-buffered saline (PBS) and scraped in 500 µl lysis buffer containing 50 mM HEPES, pH 7.4, 150 mM NaCl, 10% Glycerol, 1% Triton X-100, and protease inhibitor cocktail. Cell lysates were kept on ice for 20 minutes with repeated vortexing. Cell debris and nuclei were spin down for 10 minutes at 1,000 g. 25 µg of total proteins was loaded on 10% SDS-PAGE gels and transferred to PVDF membranes by electroblotting.

**Immunohistochemistry**

For immunohistochemistry of renal tissues, kidney specimens were embedded in optimal cutting temperature (OCT) compound and frozen in isopentane-cooled liquid nitrogen. Cryostat sections (7-9 µm) were fixed with 4% paraformaldehyde for 15 minutes and blocked with 1 x RotiImmunoblock (Roth, Karlsruhe, Germany) for 10 minutes. Sections were incubated with β- or γENaC antibodies alone or simultaneously with antibodies against 11-β-HSD -2 or AQP2 in 0.5% BSA / 0.04% sodium azide in PBS over night at 4°C. After washing, sections were incubated simultaneously with the secondary antibodies for 2 hours at room temperature. Nuclear counterstaining was performed by DAPI (Sigma). Sections were covered with Tris-buffered Mowiol, pH 8.6 (Hoechst, Frankfurt, Germany) and evaluated by epifluorescence microscopy including apotome technique (Zeiss, Göttingen, Germany).