Extrarenal disease signs of proximal renal tubular acidosis persist in non-acidemic Nbce1b/c-null mice

Methods Supplement

Organ Lysate Preparation

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Organ lysate preparation

Mice were euthanized by isofluorane overdose followed by cervical dislocation. The kidney, heart, or one hemisphere of the brain were extracted into 1 mL of ice-cold homogenization buffer (HB: 100 mM NaCl, 25 mM HEPES, 250 mM sucrose, pH 7.4) plus cOmplete Protease Inhibitor Cocktail (Sigma-Aldrich, St. Louis, MO). Organs were homogenized using a handheld D1000 homogenizer (Benchmark Scientific, Edison, NJ) and resuspended in 30 mL HB. Tissue resistant to homogenization was separated by centrifugation (1,075g for 15 min). The supernatant was transferred to a separate tube and membrane fragments were precipitated by ultracentrifugation at 41,000 g for 45 min using a Beckman Optima L-70 Ultracentrifuge (Beckman Coulter Inc., Indianapolis, IN). The resulting pellet was resuspended in 0.5 mL HB and the protein concentration determined using a BCA assay kit (Thermo).

Western blotting

5 µg/lane of organ lysate in LDS sample buffer was loaded onto a 3-8% Tris-Acetate gel alongside HiMark protein ladder (Thermo). Resolved protein was transferred onto a PVDF membrane using an XCell II Blot Module with Invitrolon PVDF Filter Paper Sandwiches (Thermo). The PVDF was incubated overnight in TBS containing 0.1 % Tween-20 and 5% milk powder and probed for NBCe1 immunoreactivity using an anti-SLC4A4 rabbit polyclonal antibody ESAP14635 (#E-AB-14348: Elabscience Biotechnology Inc., Houston, TX: characterized in this study by single band of appropriate molecular weight by western blot, as well as loss of immunoreactivity from Nbce1b/c mice) followed by an HRP-conjugated goat-anti-rabbit secondary antibody (MP Biomedicals, Solon, OH). Immunoreactive protein bands were disclosed using ECL2 reagent and the chemiluminescent signal was imaged using a myECL imager (Thermo). Densitometry was performed using FiJi software.[35,36]

Immunofluorescence

Mice were euthanized by isofluorane overdose followed by cervical dislocation. Their eyes were enucleated using dissecting forceps. Pairs of eyes were submerged in 4% paraformaldehyde at 4°C for 48 hours. Fixed eyes were embedded in paraffin and 5 µm sections were mounted onto glass slides either by the Histology Core Facility at the University at Buffalo or by the Pathology Facility at Buffalo General Medical Center.

Sections were deparaffinization via a succession of 3 min washes in xylene (twice), 1:1 xylene/100% ethanol mix, 100% ethanol (twice), 95% ethanol, 70% ethanol, 50% ethanol, and distilled water. Antigen retrieval was performed by bathing slides for 40 min in a 95°C demasking solution (1.21g Tris Base, 0.37g EDTA, 1L H_2O) and then cooling to room temperature. Slides were stored in TBS at 4°C until use.

After blocking with Rodent Block M (Biocare Medical, Pacheco, CA), NBCe1 immunoreactivity was probed using the anti-NBCe1 antibody ESAP14635 (Elabscience Biotechnology Inc.) followed by an Alex488-conjugated goat-anti-rabbit secondary antibody A-11034 (Thermo). The labelled tissue was fixed beneath a coverslip using Vectashield mounting medium with DAPI (Vector Laboratories, Burlingame, CA). Images were acquired using an Axio Imager 2 fluorescence microscope (Carl Zeiss Inc., Thornwood, NY).