**Protocol – Animal Experiments**

*Anaesthesia and preparation*

Adult male C57BL/6 mice (Taconic) weighing 25-30g were used.Anesthesia was induced with 4% isoflurane (Schering-Plough Animal Health) in a closed container, and maintained with 1.6-1.8% isoflurane in air delivered via a mask until a tracheostomy and endotracheal intubation was performed. Animals were mechanically ventilated (Ugo Basile Animal Ventilators) to an end-tidal CO2 concentration of 4.5 - 6 kPa using a volume-controlled mode (tidal volume 6 ml/kg) and a positive end-expiratory pressure of 5 cmH2O. Body temperature, measured rectally, was maintained at 37°C. The left femoral artery was cannulated for measurement of arterial pressure and blood sampling. Following cannulation of the internal jugular vein a 6% dextran solution (Meda) at a dose of 3.6 uL/g was administered during 20 min. A maintenance infusion of Ringer´s Acetate was then started at a rate of 0.3 µL/min. Animals were then received treatments as described in the methods.

*Histology of kidneys*

Tissue samples for histology were fixed in buffered 4% formalin (pH 7.4) at room temperature for 7 days after which the samples were transferred to 70% ethanol. Following dehydration samples were imbedded in paraffin (Histolab Products AB), cut into 4 µm sections, and mounted. After removal of the paraffin, tissues were stained with Mayers hematoxylin (Histolab Products AB) and eosin (Surgipath Medical Industries, Inc.).

*Immunohistochemistry of kidneys*

Paraffin-embeded samples were cut into 4 µm sections, and mounted. After rehydration and antigen retrieval, rabbit antibodies against albumin (1:1000) were added. Anti-rat albumin antibody, provided kindly by Bo Åkerström, was prepared from rabbits by immunization with rat albumin, purified from rat serum as described (Åkerström et al., 1985), and previously validated for cross-reactivity to mouse albumin (not shown). Bound antibodies were detected using horseradish peroxidase-conjugated secondary goat anti-rabbit and visualized using 3,3-diaminobenzidine as chromogen. The sections were counterstained with Mayer’s hematoxylin and mounted in Pertex.

At least five images from the cortex area of each section were obtained and the percentage of pixels with “positive” and “high positive” intensity was determined by the IHC Profiler plugin for Image J.

*Electron microscopy of kidneys*

Tissue samples for electron microscopy were fixed in 0.15 sodium cacodylate, 2.5% glutaraldehyde, pH 7.4. After fixation, samples were washed, dehydrated, critical point dried, and sputtered with palladium/gold as described in more detail earlier (Herwald et al., 2003). Specimens were examined in a Philips/FEI XL-30 field emission scanning electron microscope (FESEM) operated at an acceleration voltage of 5 kV, working distance of 7,5 mm and a magnification of 500 – 5000 times.

**References**

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