# IMMUNOHISTOCHEMICAL EVIDENCE FOR $H^+$-$K^+$-ATPase IN THE PROXIMAL TUBULE SEGMENT PIIb AND LATE DISTAL TUBULE IN THE KIDNEY OF SQUALUS ACANTHIAS.

Hartmut Hentschel[1], Peter Herter[1], Fricke Pietruschka[1] and Marlies Elger[2]
[1]Max-Planck-Institut für Systemphysiologie, D-4600 Dortmund 1, FRG
[2]Institut für Anatomie und Zellbiologie, Universität Heidelberg, D-6900 Heidelberg, FRG

Functional and immunohistochemical studies of distal tubule and collecting duct of rat, rabbit, frog and Necturus gave evidence for an ATPase which is stimulated by potassium [Wingo and Straub, J. Clin. Invest., 84:361-365, 1989; Wingo et al., Kidney Int., 38:985-990, 1990; Doucet and Marsy, Am. J. Physiol., 253:F418-F423, 1987; Cheval et al., Am. J. Physiol. 260:F800-F805, 1991; Planelles et al., Am. J. Physiol. 260:F806-F812, 1991]. The renal enzyme is insensitive to ouabain, inhibited by omeprazole, vanadate and imidazopyridine Sch28080, thus resembling the gastric $H^+$-$K^+$-ATPase. In the mammalian kidney the enzyme is thought to be located mainly at the apical cell membrane of a subset of the epithelial cells of the collecting duct, being involved in active proton secretion and potassium reabsorption. Consequently, immunohistochemical evidence has been presented for the presence of $H^+$-$K^+$-ATPase in intercalated cells of the cortical collecting duct (CCD) and the outer medullary collecting ducts (OMCDo) [Wingo et al., Kidney Int., 38:985-990, 1990; Brown et al., Kidney Int. 37:560A, 1990; Bastani et al., JASN 3:772, 1992].

We have recently localized binding sites of a polyclonal antibody against a portion of rat gastric $H^+$-$K^+$-ATPase in European dogfish, Scyliorhinus caniculus [Hentschel et al., Verh. Dtsch. Zool. Ges. 85.1:206, 1992; Hentschel et al., Pflügers Arch. Europ. J. Physiol. 420:R72, 1992; Hentschel et al., Anat. Rec., in press]. In contrast to the mammalian and amphibian kidney, in dogfish immunoreactivity for $H^+$-$K^+$-ATPase was observed in the proximal tubule (segments PI and PII) in addition to the late distal tubule. Immunoreactivity with antibody against a portion of the $\alpha$-chain of hog gastric $H^+$-$K^+$-ATPase was also observed in proximal and distal tubules of the skate Dasyatis sabina [Swenson et al., personal communication].

Physiological and biochemical studies of dogfish, Squalus acanthias, gave clear evidence for the involvement of gastric $H^+$-$K^+$-ATPase-like enzyme in renal acid secretion of elasmobranches [Swenson et al., Bulletin MDIBL 31:105-107, 1991].

For immunohistochemistry, tissue samples of kidney were obtained after perfusion fixation with 4% paraformaldehyde in phosphate buffer of sexually mature dogfish, Squalus acanthias [see also, Elger and Hentschel, this volume].

For the production of antibodies, a fragment of rat gastric $H^+$-$K^+$-ATPase was selected from the amino acid sequence [Shull and Lingrel, J. Biol. Chem. 261:16788-16791, 1986], presumably representing an extracytoplasmic domain of the membrane enzyme. A decapentamer corresponding to amino acids 726 to 739 in the model of Shull and Lingrel was synthetized by ICI (Cambridge, England) and this peptide was used to raise a variety of antibodies. Polyclonal antibodies were obtained from rabbits, which had been injected with 200 $\mu$g of synthetic coupled to Keyhole Limpet Hemocyanin (Cambridge Research,

England). After two boosts, the rabbits were bled and their serum was tested by enzyme-linked immunoabsorbance (ELISA) screening on peptide coated microplates.

Light microscopic immunohistochemistry was performed on cryosections with a fluorescent Biotin-Streptavidin technique. Cryosections (4 to 7 $\mu$m) were cut from prefixed tissue samples, which had been frozen onto cork plates by plunging them into liquid propane. Sections from blocks, which had been chemically fixed with formaldehyde (4%, prepared from paraformaldehyde) were initially treated with 0.2% sodium borohydride for 10 min. Thereafter, the same protocol was applied to sections from prefixed as well as native frozen material. Sections were exposed for 15 min to a solution of glycine in phosphate buffered saline (PBS). This step was followed by further blocking of unspecific binding sites with two changes of gelatin in PBS for 15 min each. The sections were incubated for 1 hour with antibodies against $H^+$-$K^+$-ATPase fragment. Following a thorough rinse with PBS, a second antibody against rabbit serum conjugated with tetramethyl-rhodamine-isothiocyanate (TRITC) (Dianova, Jackson laboratories) was applied for 1 hour. After a final rinse with PBS, the sections were covered with antifading (0.1% p-phenylene diamine in PBNS) and coverslips. The preparations were studied with a Leitz Orthoplan microscope using Ploemopak epifluorescence. Proximal and distal tubule segments were identified by their location in the renal zones, by their cell size and by other morphological criteria as outlined in the contribution of Elger and Hentschel, this volume.

Marked labelling was observed at the apical membrane of epithelial cells of proximal tubule segment PIIb (brush border), and at the apical membrane of late distal tubule segment LDT, in the mesial tissue (Figs. 1 and 2).

The present results, which demonstrate binding of a polyclonal antibody against a presumed extracellular domain of the transmembrane gastric $H^+$-$K^+$-ATPase are the first to localize this transport enzyme in the kidney of spiny dogfish, Squalus acanthias at the cellular level. In contrast to the mammalian kidney we obtained evidence for the presence of $H^+$-$K^+$-ATPase-like protein not only in the LDT, but also in proximal tubule segments. This distribution of binding sites resembles that observed in European dogfish. Moreover, these results corroborate the in vivo observations with Squalus acanthias by Kempton [J.Morphol. 23:247-263, 1943] and with Raja erinacea by Deetjen and Maren [Pflügers Arch. 346:25-30, 1974] of proximal and distal sites of acidification in the elasmobranch kidney.

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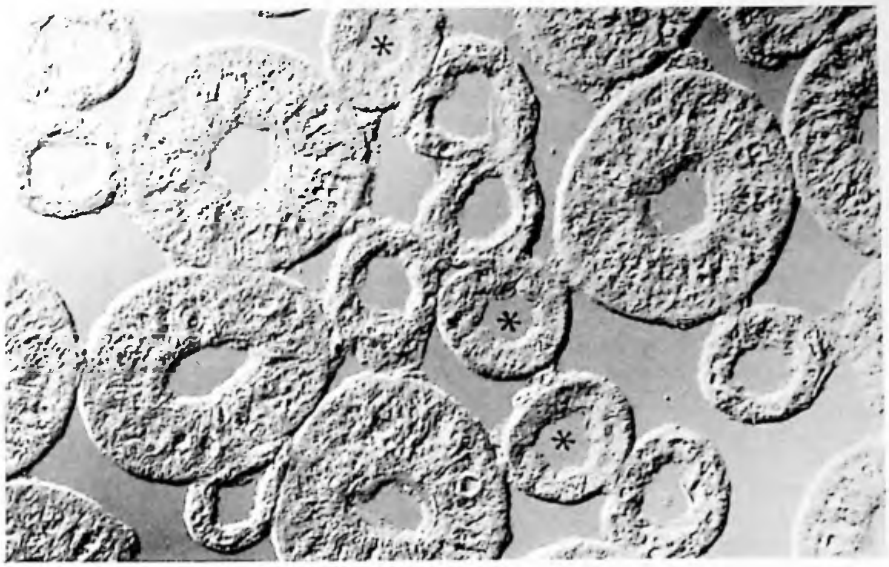

Figure 1. Cryostat section through the mesial tissue of kidney of Squalus acanthias. Differential interference contrast. Large tubules with luminal brush border and small tubules with brush border and luminal flames of cilia belong to the proximal tubule segments PIIa and PIIb, respectively. Small tubular profiles without brush border and ciliated cells (asterisks) are cross sections through the late distal tubule LDT. x500.

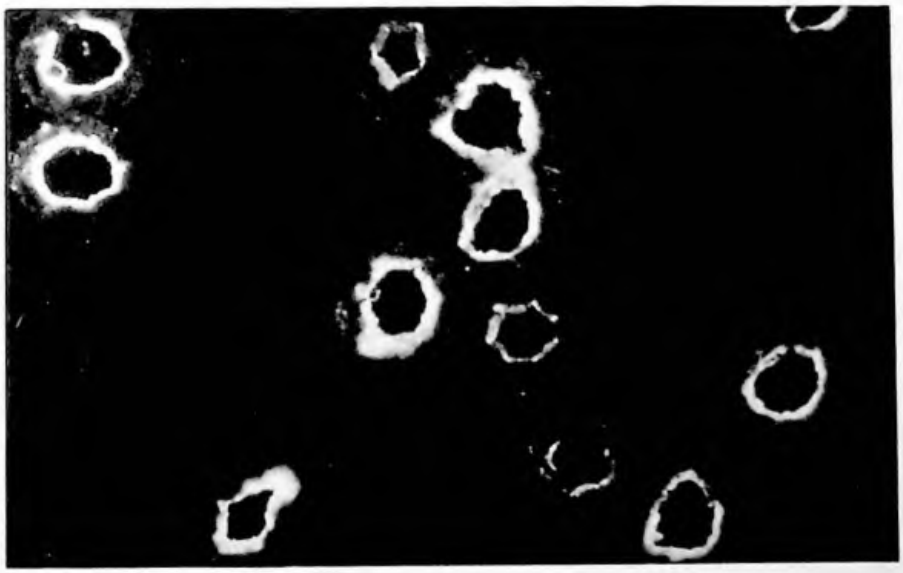

Figure 2. Fluorescence micrograph of the same preparation as in Fig. 1. Antibody against $H^+$-$K^+$-ATPase binds to the apical cell membrane of PIIb and LDT, as is demonstrated by the selective binding of antibody-fluorochrome conjugate.