FURTHER STUDIES OF IMMUNOREACTIVITY OF NaP_i-II RELATED PROTEIN IN RENAL TUBULES OF FLOUNDER, *PLEURONECTES AMERICANUS*

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We continued our immunohistochemical studies of binding of antisera against NaP_i-II related protein (Kohl B et al., Am.J.Physiol. 270:F937-F944, 1996) in the kidney of flounder. By microdissection, qualitative and quantitative histology we have shown that the winter flounder displays two proximal tubule segments, a minor portion of PI and a major portion of PII. The PII merges with the collecting tubule-collecting duct system (CT/CD) (Elger M et al., Bull MDIBL 34:97-99, 1995). The CT/CD of winter flounder, as well as the urinary bladder of this species, were considered to represent an equivalent of distal renal tubule (Gamba G et al., J. Am.Soc.Nephrol. 2:738, 1991). Phosphate transport by distal renal tubule cells has been a matter of controversy, however, mRNA of NaP_i-cotransporter was detected recently in rat renal collecting tubule cells (Custer M et al., Am.J.Physiol 266:F767-F774). In flounder, reverse transcription-polymerase chain reaction with cells from microdissected tubule portions revealed mRNA of NaP_i-II in the CT as well as in the PII (Elger M et al., in preparation).

For immunohistochemistry, kidneys of winter flounder were fixed by vascular perfusion via the bulbus arteriosus with 2.5% formaldehyde, 0.1% glutaraldehyde and 0.2% picric acid in Sörensen's buffer, pH 7.4 (Hentschel H, Am.J.Anat. 190:309-333, 1991). Polyclonal antisera and immunohistochemical protocols have been described previously (Kohl B et al., Am.J.Physiol. 270:F937-F944, 1996). Antiserum AB 33 labelled PII at the basolateral cell region, as reported previously, and antiserum AB 55 bound to the basolateral as well as the apical region of PII cells (Figures a and b). Specific binding of AB 55, which was abolished by incubation in the presence of antigenic peptide, was also observed in CT/CD cells, predominantly at the apex (Figures a and c). Glomeruli and PI were not labelled by AB 33 or AB 55. These results localize renal tubular P_i secretion of teleosts to the PII and suggest modulation of urinary Pi content by the subsequent CT/CD system.

Whereas renal handling of P_i by glomerular filtration and reabsorption in the proximal tubule, mediated by an apical NaP_i-cotransport system, generally is thought to constitute major mechanisms in the mammalian kidney, we document that in flounder already the prerequisites exist of an additional mode of phosphate control. We believe that proximal secretion and distal reabsorption of P_i are not only of adaptational value for marine fish, but may also be operative in higher vertebrates under extreme physiological or pathological conditions.

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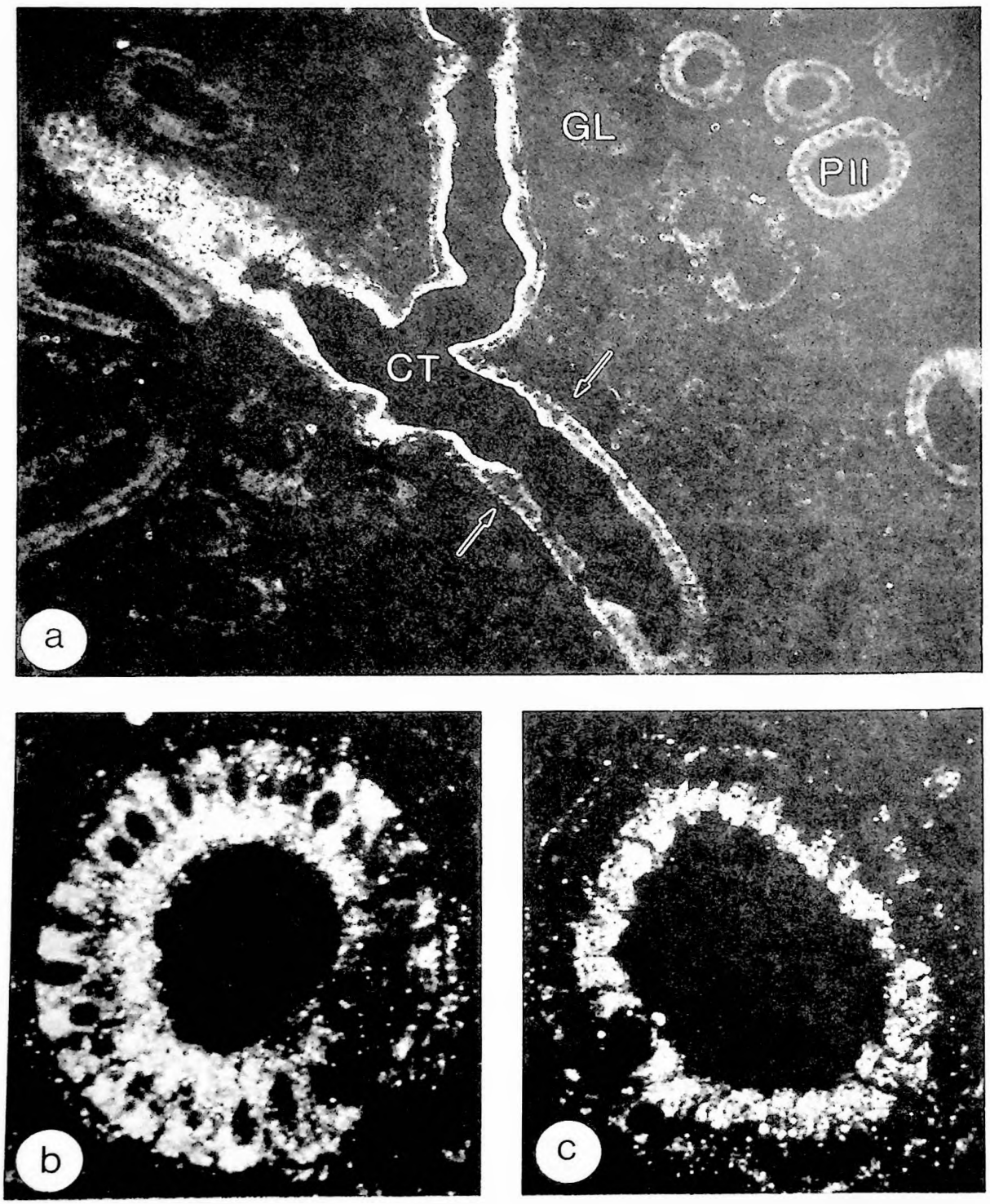
Figures: Immunohistochemical demonstration of NaPi-II related protein. Pronounced binding of AB 55 is seen at the apex of collecting tubules (CT) (a and c), and at the basolateral side, as well as at the apical region of proximal tubule PII cells (a and b). Glomeruli (GL) are negative, PI not shown. Arrows mark transitions of PII to CT. a: Conventional epifluorescence micrograph. x 250. b (PII) and c (CT): Confocal laser scanning micrographs. x 1000.


GL
PII
CT
a
b
c