IMMUNOHISTOCHEMICAL LOCALISATION OF A NaPi 2-COTRANSPORT SYSTEM IN THE KIDNEY AND INTESTINE OF WINTER FLOUNDER (PLEURONECTES AMERICANUS)

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After the cloning of a renal Na/Pi 2-cotransport system from winter flounder (Werner A, et al. Am. J. Physiol. 267: F311-317, 1994) we recently investigated by immunohistochemistry the distribution of this transport protein along the nephron. A further question of interest was whether the renal Na/Pi-transporter is also present in flounder intestine.

Winter flounder were caught in the Gulf of Maine in November 1994 and July 1995. Kidney and intestine were fixed by dripping a chilled mixture of 2% paraformaldehyde and 0.5% picric acid in 80% ethanol on the exposed organs or by perfusion with 4% paraformaldehyde. Blocks of tissue were dehydrated via graded ethanol and embedded in paraffin. For immunodetection deparaffinized sections (7 µm) were incubated with two antisera raised in rabbits against different partial sequences of the transport protein. Blocking of unspecific binding sites was performed for 15 min. with 50 mM glycine in PBS and 20 min. in PBS containing 5% goat serum, 0.2% gelatine and 0.5% BSA. Incubations with preimmune serum served as controls. Primary antisera and preimmune serum were diluted 1:400 in PBS with 0.2% gelatine and 0.5% BSA (PBG) and incubated for 2 hours with the sections. As detection system a secondary goat anti rabbit IgG antibody conjugated to Cy 3 (DIANOVA, Hamburg, Germany) diluted 1:100 in PBG was used. In double labeling experiments with Lens culinaris agglutinin (LCA) the lectin was diluted 1:50 in PBG.

We observed distinct binding of antisera specific for the Na/Pi -cotransport protein at the region of basolateral membranes of epithelial cells of the proximal tubule segment PII (Fig. 1a). Labelling was absent in controls with omission of the primary antisera or after incubation with preimmune serum. By electron microscopy of thin sections of flounder kidney, it was revealed that the basolateral cell membranes are greatly amplified by stacks of intracellular infoldings in the subnuclear cytoplasm (personal unpublished results). Our light-microscopic observations suggest sorting of the antigene to this region, as shown by the comma-shaped reaction product. Moreover, the NaPi transporter apparently is sorted differently in the flounder as compared to the mammalian proximal tubule (Murer H., NIPS 10:287, 1995). The identification of the two segments PI and PII on histological sections was possible after we had screened a variety of plant lectins for their affinity to flounder kidney structures (for methodology of lectin histochemistry see Hentschel et al., Bull MDIBL 34:32-35, 1995). Our results with the lectins revealed that Lens culinaris agglutinin (LCA) selectively binds to the brush border of PI in Winter flounder (Fig. 1b).

In flounder intestine labeling with the Na/Pi antisera could be detected in the brush border region and the subapical cytoplasm of the enterocytes (Fig. 2). The same sodium transport system seems to be present both in flounder kidney and intestine, however at different cell poles. The presumed presence of the transporter in basolateral membranes of the PII-segment - a predominantly secretory epithelium - suggests an involvement of the renal system in phosphate secretion whereas in intestine it might be involved in phophate absorption.

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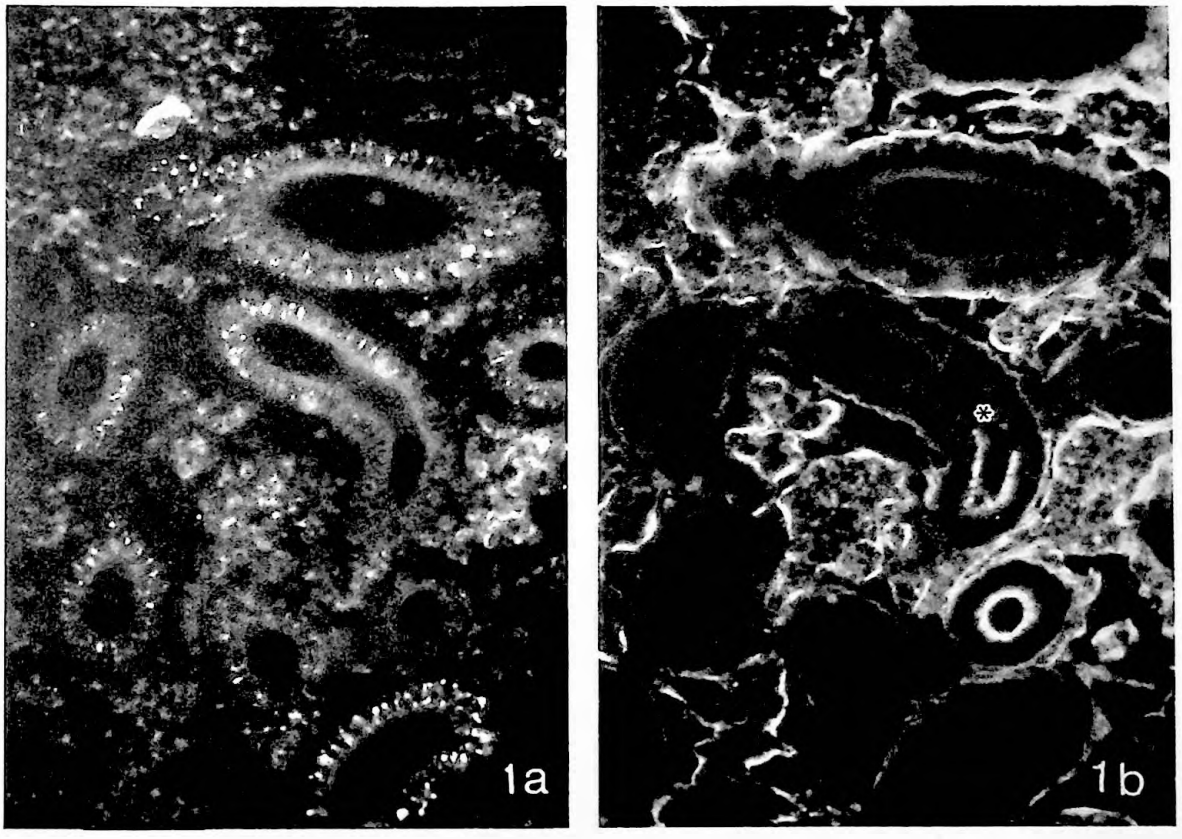

Fig. 1a Localization of the Na/Pi 2-cotransporter in the PII segment of the proximal tubule by indirect immunofluorescence. Epithelial cells of the PII-segment show immunoreaction at their basolateral membranes.

Fig.1b Identification of proximal tubule PI-segments by staining with FITC-LCA. Lectin binding sites are located at the brush border of PI-cells and at the endothelial cells of the venous sinuoid capillaries. A transition of PI to PII is marked with an asterisk.

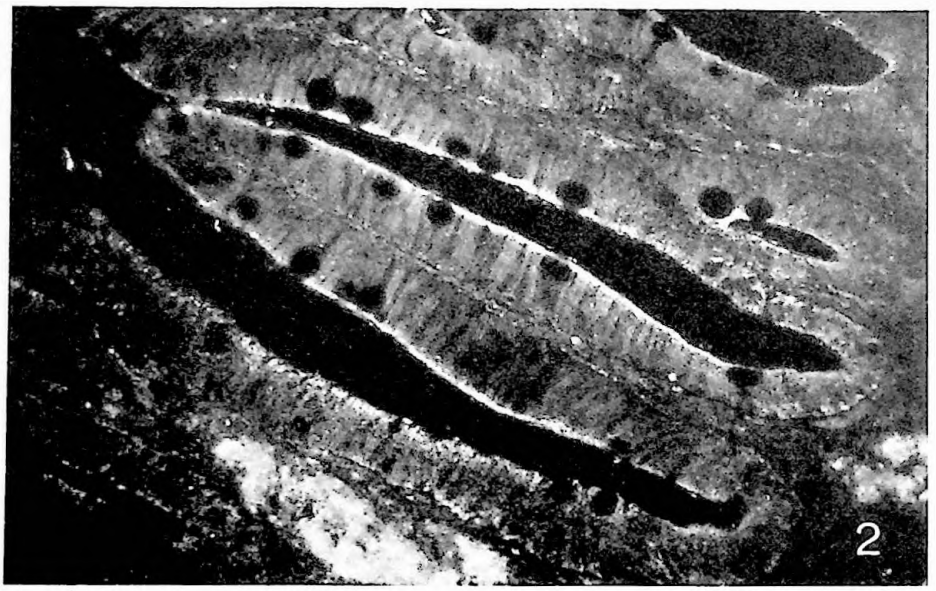

Fig. 2. Localization of the Na/Pi 2-cotransporter in the intestine of the winter flounder by indirect immunofluorescence. A marked labelling is present at the brush border region and the subapical cytoplasm of the enterocytes. The goblet cells are negative.