HELIX POMATIA AGGLUTININ IS A MARKER FOR THE END PORTION OF THE PROXIMAL NEPHRON IN SPINY DOGFISH, SQUALUS ACANTHIAS

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Plant lectins (agglutinins) bind to glycoprotein moieties on histological sections. By their sugar specificity the different lectins are accepted tools for histochemical demonstration of e.g. heterogeneous distribution of glycoproteins in tissues and organs. For instance in the kidney, a variety of lectins selectively label cells of defined segments. Dolichos biflorus agglutinin (DBA) binds to collecting ducts in rabbit kidney, peanut agglutinin (PNA) binds to a subpopulation of intercalated cells in the rabbit as well as to cells of thin limbs of Henle, and Ulex europaeus I agglutinin UEA-I specifically marks late distal tubules of European dogfish, Scyliorhinus caniculus [for a survey of literature see e.g Hentschel and Walther, Anat. Rec. 235, 21-32, 1993].

Sexually mature males of spiny dogfish, Squalus acanthias, were pithed and the kidneys were fixed with 4 % paraformaldehyde in phosphate-buffered saline (PBS, triple strength, osmolarity: 900 mOsmol/l) by vascular perfusion via the dorsal aorta. Cryostat sections (6 to 10 μm) and sections from tissue samples embedded in paraffin (8 μm) were used for binding studies with lectin-fluorochrome conjugates, as was described previously [Hentschel and Walther, Anat. Rec. 235, 21-32, 1993]. In brief, sections were treated with Na-borohydride for the removal of formaldehyde-induced fluorescence, unspecific binding was blocked by preincubation with PBS-glycine buffer, and the sections were incubated with a variety of lectin fluorochromes. Lectins and their nominal specificity for sugars are listed in Table 1. For the nomenclature and morphological criteria of nephron segments and other renal structures see Elger and Hentschel, this bulletin. The results are summarized in Table 2. Two lectins (PNA and UEA-I) did not bind, three lectins (RCA-I, SBA, and WGA) labeled only nephron segments to differing degree, one lectin (LEA) exhibited ubiquitous bindings (Fig. 1). DBA selectively labeled single cells in the early distal tubule and collecting duct.

Table 1
Lectins used and their nominal specificities

Lectin	Nominal specificities[1]
Dolichos biflorus agglutinin (DBA)	α-N-acetylgalactosamine
Peanut agglutinin (PNA)	D-galactose-(ß 1-3)-N-acetylgalactosamine
Ricinus communis agglutinin I (RCA-I)	ß-D-galactose
Soybean agglutinin (SBA)	N-acetylgalactosamine
Wheatgerm agglutinin (WGA)	N-acetylglucosamine, N-acetylneuraminic acid
Ulex europaeus agglutinin I (UEA-I)	α-L-fucose
Lycopersicon esculentum agglutinin (LEA)	N-acetylglucosamine
Helix pomatia agglutinin (HPA)	N-acetylgalactosamine

[1]Goldstein and Poretz, in: The Lectins, Academic Press, 1986

Table 2
Lectin binding in kidney of Squalus acanthias

	DBA	PNA	RCA-I	SBA	WGA	UEA-I	LEA	HPA
Glomerulus	-	-	-	+	+	-	++	-
Neck segment	-	-	(+)[2]	++	+[3]	-	+++[3]	-
Proximal tubule								
PIa	-	-	(+)[2]	+++	++[3]	-	+++[3]	(+)[3]
PIb	-	-	(+)[2]	+++	++[3]	-	+++[3]	-
PIIa	-	-	(+)[2]	++	+[3]	-	+++[4]	-
PIIb	-	-	(+)[2]	+++	++++[4]	-	+++[4]	++++[4]
Intermediate segment	-	-	(+)[2]	+++	++[3]	-	+++[3]	++++[4]
Early distal tubule	+++[1]	-	(+)[2]	+++	+++[3]	-	+++[3]	-
Late distal tubule	-	-	(+)[2]	++	+	-	+++[3]	-
Collecting tubule	-	-	-	-	-	-	+++[3]	-
Collecting duct	+++[1]	-	-	-	-	-	+++[3]	-

An arbitrary scale from weak (+) to very strong labeling + + + + was used.
1) Single cells; 2) Basement membrane; 3) Apical cell membrane; 4) Plasmalemma; - : no binding observed.

Incubation of cryostat sections and sections from paraffin-embedded tissue with Helix pomatia lectin fluorochromes resulted in strong, highly selective binding as revealed by fluorescence microscopy (Figs. 2b and 4). Two segments at the end of the proximal nephron, namely proximal tubule segment PIIb in the mesial tissue and intermediate segment in the lateral bundle were stained with this lectin-fluorochrome. Presently, it is not known, what the functional role of PIIb and IS might be. Immunocytochemistry [Hentschel et al., this bulletin] demonstrated binding of a polyclonal antibody against a portion of rat gastric H^+-K^+-ATPase to apical membranes of PIIb cells, which may indicate involvement of this segment in the acidification of the tubule content. Further studies will be performed to test the suitability of HPA for immunodissection-like enrichment of PIIb cells from isolated tubules and/or cell suspensions, e.g. with use of coated microbeads.

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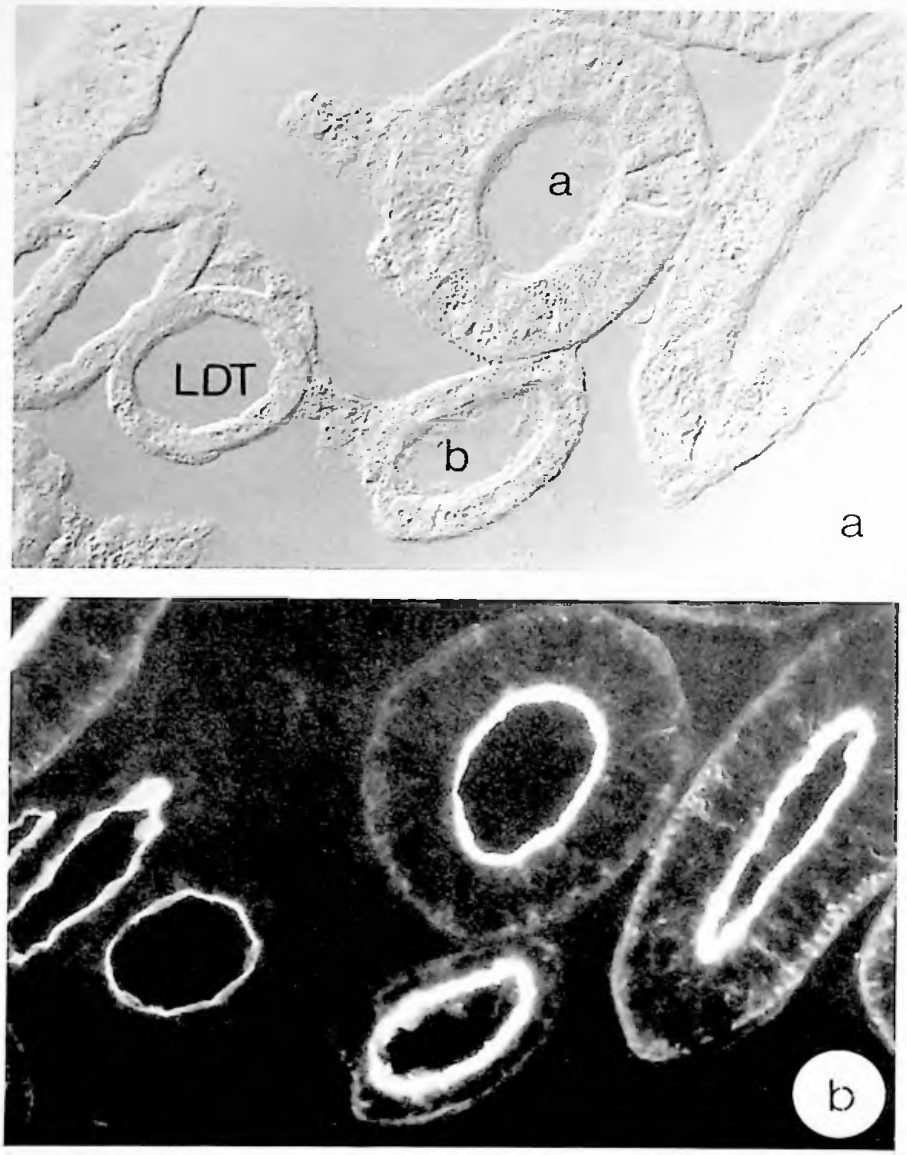

Figure 1. Mesial tissue of Squalus acanthias.

a) Differential interference contrast. Segments PIIa and PIIb have a luminal brush border and flames of cilia.

b) Fluorescence micrograph demonstrating binding of LEA-FITC at the plasmalemma of proximal tubule segments PIIa and PIIb. In the late distal tubule the fluorochrome marks the apical membrane. x 500

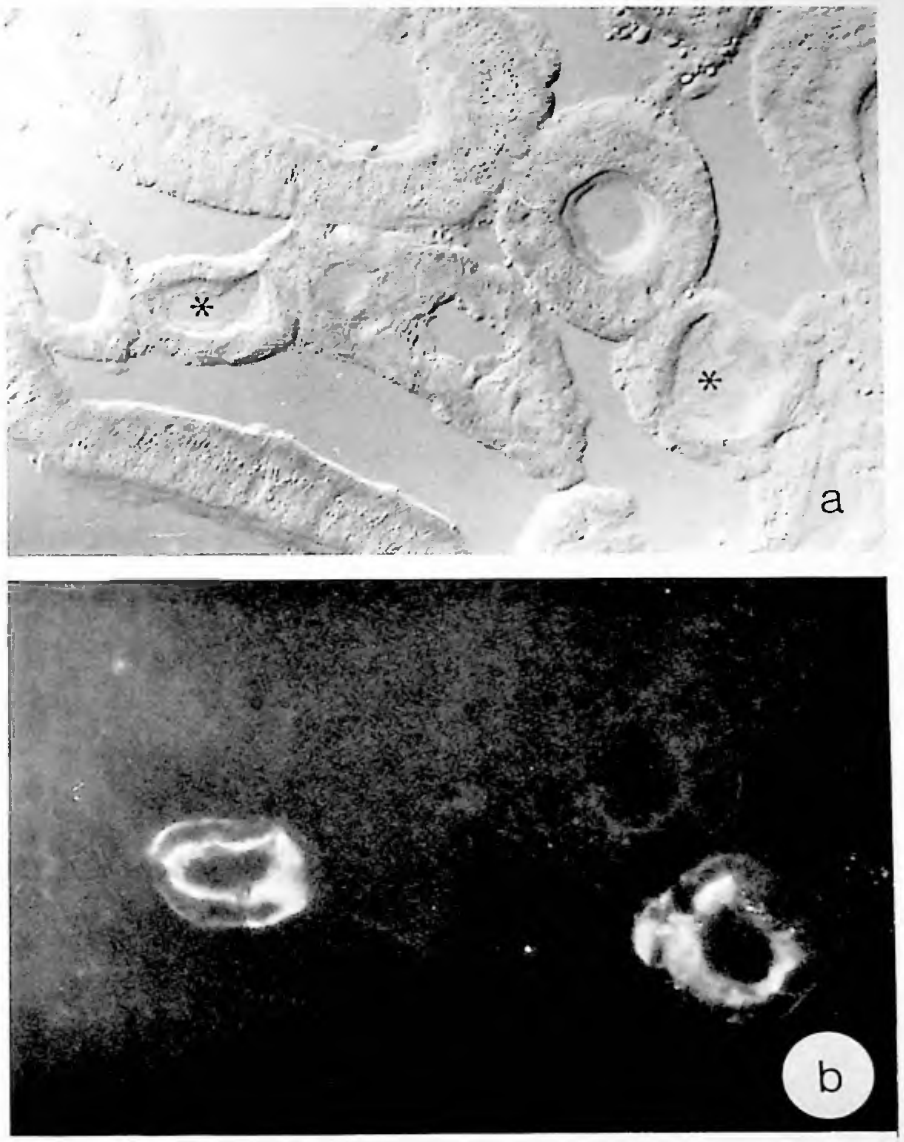

Figure 2. Mesial tissue of Squalus acanthias.
a) Differential interference contrast. PIIb is marked with asterisks.
b) Fluorescence micrograph demonstrating selective binding of HPA-FITC at the plasmalemma of PIIb cells. x 500

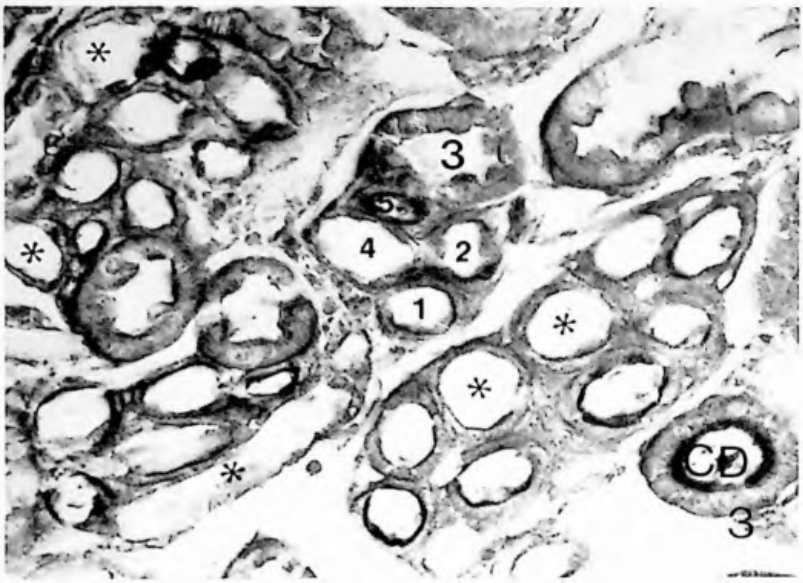

Figure 3. Cryostat section through the lateral bundle zone of Squalus acanthias. Several bundles are sectioned. Tubules are numbered in one cross section: 1 = neck segment, 2 = proximal tubule segment PIa, 3 = early distal tubule, 4 = intermediate segment, 5 = collecting tubule. CD collecting duct. Additional sections through intermediate segments are indicated by asterisks. Alcian blue-PAS. x 500

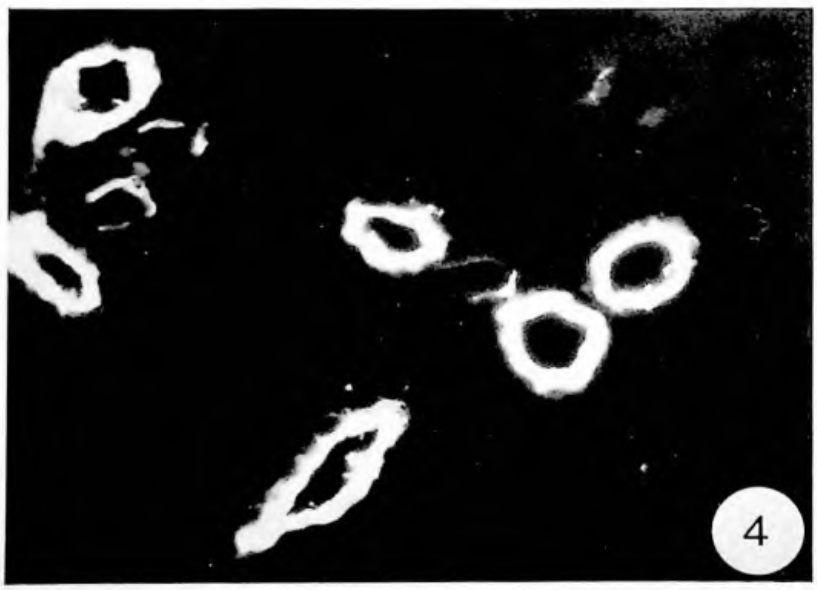

Figure 4. Fluorescence micrograph of a parallel section of the section shown in Fig. 3. Incubation with Helix pomatia agglutinin-fluorochrome. Cross sections of intermediate segments exhibit a distinct labeling. In addition, faint staining of PIa cells occurs. x 500