ENZYMATIC COMPOSITION AND TRANSPORT PROPERTIES OF BRUSH BORDER MEMBRANES ISOLATED FROM THE KIDNEY OF THE TOADFISH (OPSANUS TAU)

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The proximal tubule of glomerular kidneys simultaneously reabsorbs and secretes a variety of solutes. In the kidneys of aglomerular fish, on the other hand, almost exclusively secretion takes place. It was therefore of interest to isolate brush border membranes from aglomerular toadfish kidneys and to compare their enzymatic composition and transport properties with those described previously for brush border membranes obtained from the glomerular flounder kidney.

Toadfish (200-600 g body weight) were obtained from the Biological Laboratory in Woods Hole. The kidneys of two animals (of either sex) were used for each experiment. The tissue (7-10 g wet weight) was minced in a small volume of flounder Ringer's, tubules and hematopoetic tissue were separated and the brush border membranes were isolated by a calcium precipitation method using 15 mM $CaCl_2$, as described for flounder kidney (Eveloff et al., Am. J. Physiol. 237:F291-F298, 1979). Protein content, enzyme activities and transport of solutes were determined as detailed previously (Eveloff et al., J. Comp. Physiol. 135:175-182, 1980).

1. Enzymatic composition

In Table 1 are compiled the enzyme activities found in the tubule homogenate and in the brush border membrane fraction of the toadfish kidney. The highest enrichment was found for γ-glutamyltranspeptidase, followed by alkaline phosphatase and Mg-ATPase. Na-K-ATPase was only insignificantly enriched in the membrane fraction. The γ-glutamyltranspeptidase and the alkaline phosphatase activity characterise this membrane fraction as luminal since in a variety of tissues these enzymes are exclusively localized in the brush border membrane (Tate and Maack, in: Renal Biochemistry, R. Kinne (ed.), Elsevier, Amsterdam-New York-Oxford, 1985, pp. 63-98). The low Na-K-ATPase activity in this membrane fraction indicates that as in the glomerular kidney the active transport step for sodium in the cells of the aglomerular kidney is localized in the basal-lateral plasma membranes. Aminopeptidase M which is usually also found in brush border membranes of glomerular kidneys (Tate and Maack, as above) is absent from the toadfish brush border membrane. This result supports the assumption that the main functional role of this enzyme is the hydrolysis of peptides present in the primary urine generated by filtration in the glomerulum. Since no filtration occurs in the aglomerular kidney a lack of enzyme activity would be expected.

Table 1.-- Enzyme activities in toadfish kidney brush border membranes

	tubule homogenate	brush border membranes	enrichment
γ-glutamyltranspeptidase	14.8 ±5.5	145.6 ±44	(9.8)
alkaline phosphatase	1.5 ±0.3	9.1 ±2.0	(6.1)
Na-K-ATPase	2.5 ±1.3	3.7 ±1.4	(1.5)
Mg-ATPase	10.3 ±2.5	50.8 ±9.9	(4.9)
aminopeptidase M	not detectable		

Enzyme activities are given as specific activities in μmoles/h x mg protein, they represent mean values ± S.D. derived from at least 6 experiments. Enrichments of enzymes defined as the ratio between the specific activity of the enzyme in the membrane fraction and the specific activity in the homogenate are given in parentheses.

2. Transport properties

Table 2 shows the uptake of D-glucose, L-alanine and L-taurine by toadfish kidney brush border membranes. When the uptake through sodium cotransport systems is compared (by calculating the phlorhizin-inhibitable or sodium-dependent transport) L-taurine uptake is about two times higher than D-glucose uptake and about 1.5 times higher than L-alanine uptake. Table 2 also gives in parentheses the transport activities found in the brush border membrane of flounder kidneys. The transport rates for taurine are similar in the two membranes whereas D-glucose transport and L-alanine transport in the flounder kidney brush border are at least tenfold higher than in the toadfish brush border. This result cannot be explained by the higher intravesicular space in the flounder brush borders (about 2.5 times higher) and therefore suggests differences in transport activities. The difference is most evident when the ratios taurine/glucose and taurine/alanine are compared. In the toadfish the ratio with regard to glucose is 20fold higher and the ratio with regard to alanine is about tenfold higher.

Thus, although V_{max} values have not been determined, transport systems involved in reabsorption such as the D-glucose transport system and the L-alanine transport system exhibit a lower activity in the brush border membranes of a nonfiltering kidney than of a filtering kidney. The transport systems are, however, not completely absent and probably reabsorb substrates entering the tubule through leak pathways. On the other hand, the taurine sodium cotransport system represents a major transport activity in the brush border of the aglomerular kidney. These results strongly support our hypothesis (King et al., J. Comp. Physiol. B 155:185-193, 1985) that sodium-taurine cotransport across the luminal membrane is involved in taurine secretion by the proximal tubule.

Table 2.-- Sodium cotransport systems in toadfish kidney brush border membranes

	Solute uptake after				
	15 s	60 s	105 s	120 min	n
D-glucose					
75 mM NaCl	25.5±3.2	43.1±7.4	58.6±18.5	89.1±22.8	
75 mM NaCl + 0.1 mM phlorhizin	17.8±2.1	30.3±2.8	35.9± 3.8	80.3±12.9	3
phlorhizin-sensitive	7.7 (210)	12.8 (260)	22.7		
L-alanine					
75 mM NaCl	21.3	34.0	42.0	86.5	2
75 mM KCl	12.2	20.9	25.7	77.5	
sodium-dependent	9.1 (130)	13.1 (140)	16.3		
L-taurine					
75 mM NaCl	20.5±2.2	44.4±15.7	59.4±26.7	117.3±32.0	3
75 mM KCl	5.4±2.7	12.5±1.3	17.0±3.0	59.3±16.1	3
sodium-dependent	15.1 (20)	31.9 (34)	42.4		

Ratio	taurine/glucose		taurine/alanine	
	15 s	60 s	15 s	60 s
toadfish	1.9	2.5	1.7	2.44
flounder	0.09	0.13	0.15	0.25
toadfish/flounder	21	19.3	11.3	9.8

Mean values derived from at least two experiments are given in pmoles/mg protein with the standard deviation. Values in parentheses represent the uptake by flounder kidney brush border at identical substrate concentrations (0.1 mM). For D-glucose and L-alanine a 100 mM salt gradient instead of a 71 mM salt gradient was present (Eveloff et al., J. Comp. Physiol. 135:175-182, 1980). In flounder brush border membranes uptake after 120 min equaled 180 - 210 pmoles/mg protein. n = number of experiments.