EFFECT OF CADMIUM ON EPITHELIAL TRANSPORT SYSTEMS IN PSEUDOPLEURONECTES AMERICANUS. STUDIES WITH ISOLATED PLASMA MEMBRANE VESICLES

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Sodium Cotransport Systems in the Proximal Tubule of the Flounder Kidney

In order to study the effect of cadmium on sodium cotransport systems present in the luminal membrane of the proximal tubule, brush border membrane vesicles were isolated from the kidney of winter flounders (Pseudopleuronectes americanus) by a differential precipitation method as described previously (Eveloff et al., J. Comp. Physiol. 135:175-182, 1980). Transport of solutes into the isolated vesicles was measured by a rapid filtration technique (ibid.). Enrichment of the brush border membranes was monitored by determination of the activity of the marker enzymes alkaline phosphatase and γ-glutamyltranspeptidase in the isolated membrane fraction and the tubule homogenate. The enrichment of the marker enzymes in the membrane samples investigated in this study was 7.6 for alkaline phosphatase and 11.0 for the γ-glutamyltranspeptidase. These values are similar to those reported in previous studies (King et al., J. Comp. Physiol. B 155:185-193, 1985).

Sodium-alanine cotransport

Since clinical studies indicate an impairment of amino acid reabsorption in the proximal tubule of mammals (including humans) after exposure to cadmium, first the effect of cadmium on one of the prominent amino acid-sodium cotransport systems, the sodium-alanine cotransport system was investigated.

The results of these studies are compiled in table 1. Cd significantly decreased L-alanine uptake after 15 seconds and 1 minute incubation in the NaCl but not in the KCl medium. After 90 minutes of incubation Cd lead to a slight reduction of intravesicular alanine content in both media. Under control conditions a difference between uptake in NaCl and KCl medium of 39.2 pmoles was found after 15 seconds and of 57.2 pmoles after 1 minute of incubation. In the presence of Cd these differences were 21.0 pmoles after 15 seconds and 30.8 pmoles after 1 minute. Thus Cd inhibited the sodium-dependent uptake of alanine after 15 seconds and 1 minute by about 47%. It should be noted that a substantial inhibition of sodium-L-alanine cotransport was only observed when the brush border membrane vesicles were preexposed to cadmium for 30 minutes at 15°C.

Table 1 - Effect of nominally 0.1 mM Cd on alanine uptake in flounder brush border membrane vesicles

	L-alanine uptake (pmoles/mg protein)					
	after 15 s	n	after 1 min	n	after 90 min	n
NaCl medium						
control	65.0±10.4	4	92.0±18.7	4	107.5±9.5	4
+ Cd	37.3± 9.8	4	67.5±11.6	4	86.4±2.8	4
p	< 0.005		< 0.025		< 0.025	
KCl medium						
control	16.8±3.6	3	34.8±3.9	3	90.4±14.9	3
+ Cd	16.3±2.7	3	36.7±1.5	3	69.0±15.4	3
p	n.s.		n.s.		< 0.05	

Isolated brush border vesicles were incubated for 30 minutes at 15°C in a medium containing 50 mM mannitol, 2 mM $CaCl_2$, 20 mM Hepes (pH 8.2 adjusted with Tris), and 0.1 mM $CdCl_2$. Control vesicles were preincubated in the same solution but without $CdCl_2$. L-alanine uptake was measured at 15°C in media containing in addition 12 μCi ^{3}H-L-alanine, 0.1 mM L-alanine, and 75 mM NaCl or 75 mM KCl, respectively. Uptake was terminated at given time intervals by dilution of 20 μl membrane suspension into 1 ml of an icecold "stop solution" containing 240 mM mannitol, 100 mM NaCl, 2 mM $CaCl_2$, and 20 mM Hepes (adjusted to pH 8.2 with Tris). Mean values derived from paired experiments performed in duplicate are given ± S.E.M. p values were calculated for paired data, n.s. = not significant.

In order to investigate whether the inhibition of the sodium-L-alanine cotransport system was due to a change in the transport system itself or caused by a change in the driving forces for L-alanine transport, the effect of cadmium pretreatment on another sodium cotransport system, namely the sodium-D-glucose cotransport system, was examined. As shown in figure 1 cadmium did not significantly change the uptake of D-glucose into isolated flounder brush border membrane vesicles in the early phase of the experiment. With regard to the equilibrium uptake changes similar to the ones observed with L-alanine were found.

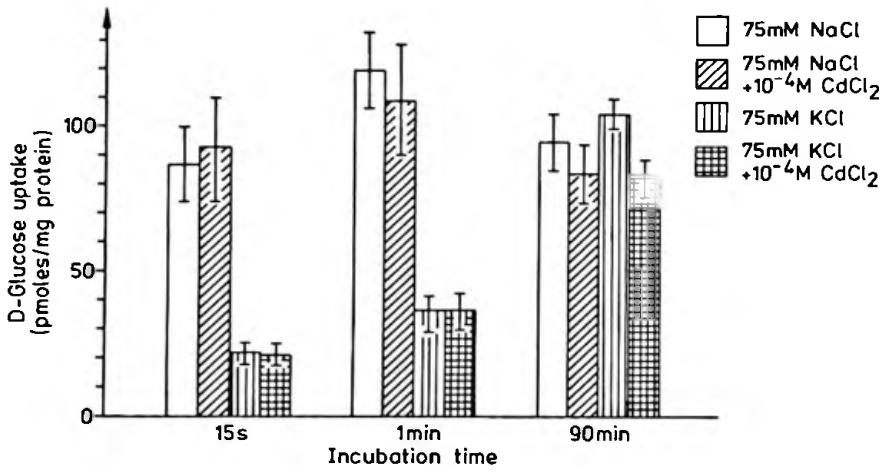

Fig. 1: Effect of cadmium on D-glucose uptake into flounder kidney brush border membranes. Conditions of the preincubation period and the uptake experiment were identical except that alanine was replaced by D-glucose in the incubation medium and that 0.1 mM phlorizin was added to the "stop solution". Mean values derived from three paired experiments performed in duplicate are given with the standard errors of the mean values.

The results presented above suggest that cadmium inhibits the sodium-L-alanine cotransport system in isolated flounder brush border membranes by a direct interaction with the transport system. The actual sensitivity of the sodium-L-alanine transport system to cadmium remains to be determined, since the free concentration of cadmium at the site of action is currently unknown.

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