CADMIUM INHIBITION OF SODIUM-ALANINE COTRANSPORT IN RENAL BRUSH BORDER MEMBRANES FROM THE WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS)

C. Bevan*, B. Chauncey+, and R. Kinne*
* Max-Planck-Institut fuer Systemphysiologie, 4600 Dortmund, F.R.G.
+ Mount Desert Island Biological Laboratory, Salsbury Cove, ME 04672, U.S.A.

Chronic exposure to cadmium can result in impairment of amino acid reabsorption in the proximal tubule of the kidney. Using brush border membrane vesicles isolated from flounder kidney, we have studied the effect of cadmium on L-alanine transport. We have previously shown (Kinne-Saffran et al., Bull. MDIBL 26:15, 1986) that pretreatment of vesicles with 0.1 mM $CdCl_2$ resulted in time-dependent inhibition of L-alanine uptake in the presence of a NaCl gradient, but not a KCl gradient. Inhibition was due to a direct interaction with the transport system and not a change in the driving force for L-alanine transport since cadmium did not affect Na-D-glucose cotransport operating in the same membranes.

To evaluate the mechanism by which Cd^{2+} inhibits Na-dependent L-alanine transport, we determined L-alanine uptake at varying L-alanine concentrations in the presence or absence of 0.2 mM $CdCl_2$. As shown in Table 1, cadmium increased the K_m and decreased the V_{max} of L-alanine transport, indicating mixed-type inhibition.

Table 1. Effect of $CdCl_2$ on the kinetics of L-alanine transport in isolated flounder kidney brush border membranes (initial rate, trans zero conditions)

	K_m μM	V_{max} nmol·mg protein^{-1}·min^{-1}	n
Control	41±7	3.9±1.1	4
0.2 mM $CdCl_2$	131±60*	1.7±0.5*	4

* $p < 0.05$

Cd^{2+} uptake by flounder kidney brush border membrane vesicles was also studied to determine the site of action of Cd^{2+} on Na^+-L-alanine cotransport. Cd^{2+} uptake showed time- and temperature-dependance. Increasing medium osmolarity (decreasing vesicle volume) by preincubation of vesicles in media containing 0, 100, 200, or 400 mM sucrose for 60 min had little effect on Cd^{2+} uptake, indicating that the process involves primarily binding of Cd^{2+} to membrane component(s). Short exposure (about 10 sec) of the vesicles to EDTA in an ice-cold "stop solution" removed only a small fraction of Cd^{2+} from the membrane vesicles. This finding seems to indicate that a large fraction of Cd^{2+} bound to the membrane vesicles is not freely accessible to complexing by EDTA. Further evidence for the binding of Cd^{2+} to internal sites was derived from experiments showing that Cd^{2+} uptake as well as Cd^{2+} efflux initiated by the addition of EDTA are temperature-sensitive. This temperature-sensitive step probably represents the transfer of Cd^{2+} across the membrane.

The mixed-type inhibition of Cd^{2+} on the sodium-dependent L-alanine transport probably results from Cd^{2+} binding to multiple sites on the transport system. This conclusion is supported by experiments in which EDTA was added to Cd^{2+}-treated vesicles to remove all externally-bound Cd^{2+} and only a partial reversal of the inhibition of Na^{+}-L-alanine cotransport was observed.

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