

EFFECT OF CADMIUM ON EPITHELIAL TRANSPORT SYSTEMS IN SQUALUS ACANTHIAS:  
STUDIES WITH ISOLATED PLASMA MEMBRANE VESICLES

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Effect of Cadmium on Transport Properties of Rectal Gland Plasma Membrane Vesicles

In order to investigate the effect of cadmium on transport systems present in rectal gland plasma membranes, plasma membranes were isolated from dogfish (*Squalus acanthias*) by differential centrifugation as previously described (Eveloff et al., *Pflügers Arch.* 378:87-92, 1978). This membrane fraction contains predominantly basal-lateral plasma membranes of the cell which possess as main transport systems the Na,K,Cl cotransporter, the barium-sensitive K channel, and the Na,K,ATPase. The activity of the Na,K,Cl cotransporter was determined by measuring the chloride-dependent uptake of rubidium into isolated rectal gland plasma membrane vesicles by a rapid filtration method reported earlier (Hannafin et al., *J. Membrane Biol.* 75:73-83, 1983). The activity of the Na,K,ATPase was investigated by determining the ouabain-sensitive release of inorganic phosphate from ATP in the presence of Mg, Na, and K. A colourimetric assay modified after Fiske-Subbarow was employed, details of the method have already been described (Eveloff et al., *Pflügers Arch.* 378:87-92, 1978; Hannafin et al., *ibid.*).

Rubidium uptake into rectal gland plasma membrane vesicles

In figure 1 the effect of cadmium preincubation (nominally 0.1 mM) on the uptake of rubidium in the presence of a sodium chloride gradient and in the presence of a sodium nitrate gradient are shown. Both fluxes seem to be affected by cadmium. Rubidium uptake in the presence of chloride decreased by an average of 32% whereas rubidium uptake in the presence of nitrate increased by 23%. Despite the large scatter both effects were significant at a  $p < 0.05$  level. In the latter experimental setup rubidium uptake represents the overall potassium permeability of the membrane since nitrate permeability of the membrane is very high and therefore rubidium uptake by the membrane vesicles is mainly determined by the rubidium permeability which closely mirrors the potassium permeability. Thus, cadmium appears to increase the potassium permeability of the rectal gland plasma membranes. To what extent this increase in potassium permeability reflects an increase of the permeability of the membranes for cations in general remains to be determined.

In view of the changes in cation permeability of the plasma membranes in the presence of cadmium it is at present not possible to decide whether the decrease of chloride-dependent rubidium uptake found after cadmium pretreatment of the membranes reflects a direct inhibition of the Na,K,Cl cotransport system or is caused by a change in the driving forces for the transport system.

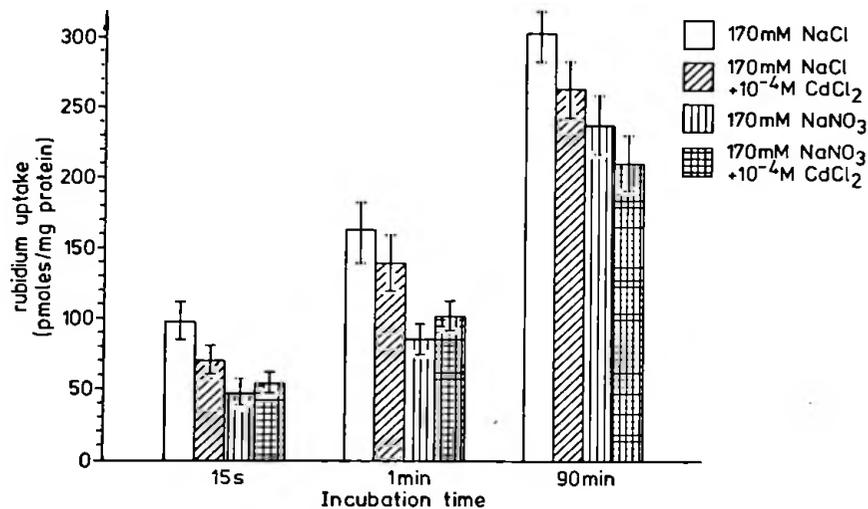


Fig. 1: Effect of cadmium on rubidium uptake by isolated rectal gland plasma membrane vesicles.

Isolated plasma membrane vesicles were incubated for 30 minutes at 15°C in a medium containing 200 mM mannitol, 1 mM Mg(NO<sub>3</sub>)<sub>2</sub>, 20 mM Hepes (pH 7.6 adjusted with Tris), and 0.1 mM CdCl<sub>2</sub>. Control vesicles were incubated in the same solution but without CdCl<sub>2</sub>. Rubidium uptake was measured at 15°C in media that contained in addition 5 μCi <sup>86</sup>Rb, 0.4 mM RbNO<sub>3</sub>, and 170 mM NaCl or 170 mM NaNO<sub>3</sub>, respectively. Uptake was terminated at given time intervals by dilution of 20 μl membrane suspension into 1 ml of ice cold "stop solution" containing 240 mM mannitol, 200 mM KNO<sub>3</sub>, 1.2 mM Mg(NO<sub>3</sub>)<sub>2</sub>, and 20 mM Hepes (pH adjusted to 7.6 with Tris). Mean values derived from five paired experiments performed in duplicate are given with the standard errors of the mean values. For the 15 seconds and 1 minute time points the differences between control and cadmium treated membranes are significant at a p level of at least < 0.05.

#### Effect of cadmium on Na,K,ATPase activity

Figure 2 compiles a dose response curve of the Na,K,ATPase activity and of the Mg,ATPase activity in isolated rectal gland plasma membranes in the presence of various cadmium concentrations. Na,K,ATPase activity is inhibited by cadmium in a dose-dependent manner with an apparent K<sub>i</sub> of 5 x 10<sup>-7</sup> M. Mg,ATPase activity of the membranes remains unaffected by cadmium even at concentrations above 1 mM.

In summary, the transport system most sensitive to cadmium found in isolated rectal gland plasma membrane vesicles is the Na,K,ATPase activity. Since in the *in vitro* system both the cytosolic and the extracellular surface of the plasma membrane are exposed to cadmium the important question from which side cadmium does interfere with the enzyme reaction remains currently unanswered. A possible sidedness of the cadmium effect would be of extreme importance for the response of the intact rectal gland

to cadmium exposure. If cadmium would act only at the cytosolic surface of the membrane, short time application of low concentrations of cadmium from the extracellular space would be expected to only barely affect the function of the rectal gland. Longterm exposure, however, during which cadmium can gain access to the cytosol should lead to an inhibition of chloride secretion of the gland since the activity of the Na,K,ATPase would be strongly reduced.

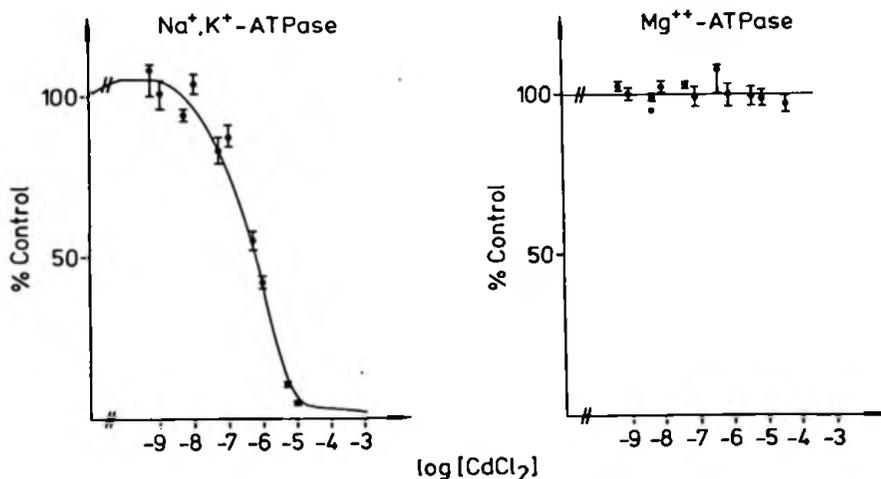


Fig. 2: Effect of cadmium on Na,K-ATPase activity and Mg,ATPase activity in isolated rectal gland plasma membranes. The membranes were preincubated with increasing cadmium concentrations in 10 mM imidazole buffer adjusted to pH 7.4 with HCl. The enzyme assay was performed in 50 mM imidazole buffer at pH 7.4. Mg-ATPase represents the ouabain-insensitive portion of the ATPase activity. Mean values derived from 4 experiments performed in duplicate are given with the standard deviations of the mean values.

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