EFFECT OF CADMIUM IONS ON PARTIAL REACTIONS OF THE NA-K-ATPASE IN RECTAL GLAND PLASMA MEMBRANES OF SQUALUS ACANTHIAS.

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Previous experiments have revealed that low concentrations of cadmium ions inhibit the Na-K-ATPase activity in dogfish rectal gland appreciably only when the heavy metal gains access to the cytoplasmic face of the enzyme [E. Kinne-Saffran et al., in: 1st Annual Report of Progress - Center for Membrane Toxicity Studies, pp. 29-34, MDIBL, 1986; P. Silva et al., in: 2nd Annual Report of Progress - Center for Membrane Toxicity Studies, pp. 29-34, MDIBL, 1987]. These results suggest that cadmium affects partial reactions of the enzyme taking place at the membrane/cytosol interface. Such reactions include the interaction with sodium, ATP and the divalent cation magnesium. We therefore studied the effect of cadmium on the kinetics of the Na-K-ATPase with regard to its sodium, ATP and magnesium dependence. We furthermore investigated whether any of these compounds could protect the enzyme from being inhibited by cadmium.

Plasma membranes were isolated from rectal glands of Squalus acanthias by differential centrifugation as source for the Na-K-ATPase activity [J.A. Hannafin et al., J. Membrane Biol. 75: 73-83, 1983]. Enzyme activity was determined by measuring the amount of inorganic phosphate released from ATP during incubation of the lyophilized membranes with the appropriate buffers, substrate, mono- and divalent cations as described previously [J.A. Hannafin et al, see above].

Analysis of the sodium dependence of the Na-K-ATPase activity in the presence or absence of 5×10^{-6} M nominal cadmium revealed no significant differences in the affinity or stoichiometry of the enzyme for sodium. This excludes the sodium binding site(s) as target site for cadmium. When the effect of varying ATP concentrations on the extent of cadmium inhibition was investigated it was observed that a reduction of the ATP concentration, keeping the nominal magnesium concentration at 6 mM, from 3 mM (the usual concentration in the enzymatic assay) to 0.5 mM shifted the dose response curve for cadmium inhibition to the right, increasing the apparent K_i value by a factor 2 to 3 (see figure 1). The inhibition pattern obtained by ranging the ATP concentration at constant Cd level suggests an uncompetitive nature of interaction. These two results taken together indicate that cadmium does not directly interact with the ATP binding site of the enzyme. The apparently decreased cadmium sensitivity of

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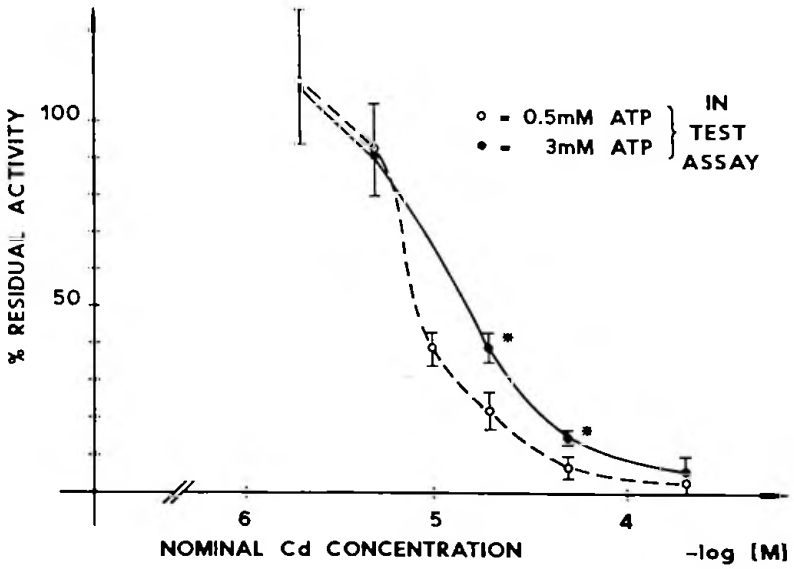

Figure 1. Effect of ATP on cadmium-dependent inhibition of Na-K-ATPase. Rectal gland plasma membranes were incubated for 30 minutes at 15°C at the nominal cadmium concentrations indicated in the figure and Na-K-ATPase activity was subsequently assayed in the presence of 0.5 mM or 3 mM ATP. Mean values ± S.D. from at least 3 determinations are given, an asterisk indicates significant differences at $p < 0.05$. Nominal concentrations have been calculated according to the amount of $CdCl_2$ added to the assay medium.

the enzyme at lower ATP concentrations can be understood by taking into account that ATP forms strong association complexes with magnesium. Thus at a lower ATP level the free magnesium concentration in the incubation medium is higher and could protect the enzyme against the inhibitory action of cadmium. In order to test this hypothesis membranes were exposed to 5×10^{-6} M nominal cadmium in the presence of varying magnesium concentrations at a constant ATP level and the extent of inactivation of the enzyme activity was recorded. As shown in table 1 increasing the magnesium concentration from 0.5 mM to 3 mM significantly decreased the extent of inactivation, suggesting a protective effect of magnesium on the enzyme.

Table 1
Effect of magnesium on inactivation of rectal gland Na-K-ATPase by cadmium

Mg concentration	% inhibition by Cd	n
0.5 mM	19.5±0.97	(3) $p < 0.001$
1 mM	7.5±0.71	(4) $p < 0.001$
3 mM	2.6±0.13	(4)

Membranes were incubated for 30 minutes at 25°C with or without nominal 5×10^{-6} M cadmium in the presence of 3 mM ATP and varying concentrations of magnesium. The rate of ATP hydrolysis was measured by determination of the P_i released during this incubation period. Mean values ±S.D. derived from n experiments are given, p values are given for the comparison with the results obtained in the presence of 3 mM ATP.

These studies suggest that one of the sites at which cadmium interacts with the Na-K-ATPase is a magnesium binding site, the occupation of which by magnesium or manganese is a prerequisite for ATP-hydrolysis. Thus the intracellular magnesium concentration might exert an important protective effect against the toxicity of cadmium in renal cells.

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