EFFECT OF CADMIUM ON K^+-STIMULATED NEUTRAL p-NITROPHENYLPHOSPHATASE IN RECTAL GLAND PLASMA MEMBRANES OF SQUALUS ACANTHIAS

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In previous studies we have shown that low concentrations of cadmium inhibit the Na^+-K^+-ATPase activity measured in isolated plasma membranes from shark rectal gland [Kinne-Saffran et al., MDIBL Bull. 26: 15-17, 1986]. In accordance with results obtained in isolated perfused rectal glands [Silva et al., in: 2nd Annual Report of Progress - Center for Membrane Toxicity Studies, pp. 60-62, 1987] it was postulated that cadmium exerts its inhibitory action at the cytoplasmic face of the membranes. Partial reactions of the enzyme taking place at the membrane/cytosol interface are the interaction with sodium, ATP and magnesium. Subsequently performed experiments showed that the sodium site was not a target site for cadmium. Increasing the ATP or the magnesium concentration reduced, however, the inhibitory effect of cadmium on Na^+-K^+-ATPase activity [Kinne-Saffran et al., MDIBL Bull. 30: 38-40, 1991]. Since ATP is a strong chelator for magnesium and cadmium these studies did not allow to differentiate between a competition of cadmium with the magnesium site of the enzyme proper or with the magnesium-ATP interaction. Therefore, the effect of magnesium and cadmium on a partial reaction of the Na^+-K^+-ATPase reaction cycle, the K^+-stimulated neutral p-nitrophenylphosphatase was investigated in the present study.

Plasma membranes were isolated from rectal glands of Squalus acanthias by differential centrifugation as source for the K^+-stimulated neutral p-nitrophenylphosphatase activity [Hannafin et al., J. Comp. Physiol. B 155: 415-421, 1985]. Enzyme activity in lyophilized membranes was determined at 15°C in the presence of 50 mmol imidazole buffer, pH 7.6, 3 mmol p-nitrophenylphosphate, 1 mmol magnesium chloride, with and without 5 mmol potassium chloride as standard incubation medium. The release of p-nitrophenolate was followed at a wavelength of 405 nm in a recording spectrophotometer. The difference between the activity in the presence and absence of K^+ was considered to represent the K^+-stimulated neutral p-nitrophenylphosphatase related to the Na^+-K^+-ATPase in these membranes.

Analysis of the effect of cadmium on the K^+-stimulated neutral p-nitrophenylphosphatase activity revealed that the inhibition of cadmium increased with time reaching a maximum after 30 minutes of incubation at 15°C. Without preincubation the apparent K_i of cadmium was 8.9×10^{-6} mol. The K_i after preincubation of the membranes was 2.2×10^{-6} mol, thus a 4-fold increase in sensitivity of the enzyme was observed. It was also found that the degree of inhibition by various cadmium concentrations was strongly dependent on the concentration of magnesium present during the preincubation period. When the dose response curve in the presence of 1 mmol magnesium was compared with the dose response curve in the presence of 10 mmol magnesium a shift to the right was observed, which resulted in a decrease in the apparent K_i in the presence of 10 mmol magnesium to 1.4×10^{-5} mol cadmium. This represents a 6-fold decrease in sensitivity of the enzyme against cadmium inhibition (figure 1). Similarly, at a constant cadmium concentration increasing magnesium concentrations from 0.5 mmol to 5.0 mmol led to an attenuation of the inhibition from 40% to only 18%.

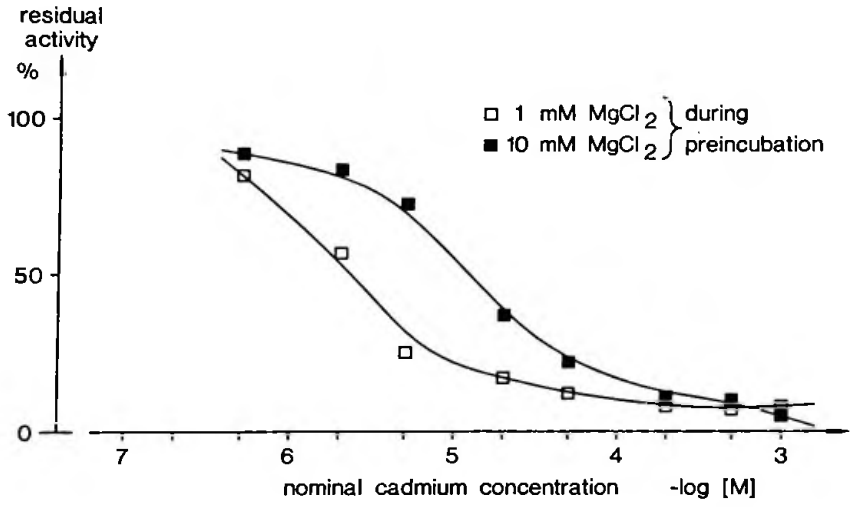

Figure 1. Effect of magnesium on cadmium-dependent inhibition of K^+-stimulated neutral p-nitrophenylphosphatase. Lyophilized rectal gland plasma membranes were incubated for 30 min at 15°C at the cadmium and magnesium concentrations indicated in the figure and the enzyme activity was subsequently assayed in the presence of 1 mM or 10 mM magnesium. Mean values from 3 determinations are given. Nominal concentrations have been calculated according to the amount of cadmium chloride added to the assay medium.

In order to further investigate the mode of action of cadmium on the K^+-stimulated neutral p-nitrophenylphosphatase we studied the effect of cadmium on the affinity of the enzyme towards magnesium. In the absence of cadmium a K_m for magnesium of 0.88 ± 0.29 mmol was found. In the presence of 4×10^{-5} mol cadmium the K_m value doubled to 1.73 ± 0.33 mmol, whereas the maximum velocity was only reduced by about 25% (167 ± 32 µmol/min vs. 140 ± 16 µmol/min; n = 3).

These studies suggest that one of the sites at which cadmium interacts with the Na^+-K^+-ATPase in shark rectal gland plasma membranes is a magnesium binding site which plays an essential role in initiating ATP hydrolysis. Since it is assumed that these magnesium sites are located at the cytoplasmic face of the enzyme molecule the intracellular magnesium concentration may play an important role in modulating the cellular toxicity of cadmium. Thus, high intracellular magnesium concentrations could exert a protective effect against the toxicity of cadmium in renal cells.

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