EFFECT OF MERCURIC CHLORIDE ON TRANSPORT IN THE RECTAL GLAND OF SQUALUS ACANTHIAS

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We have previously postulated that Na-K-ATPase is not the site of inhibition by mercury of chloride secretion by the rectal gland (Silva, P. et al. Bull. MDIBL 1994, 33:79) even though mercury inhibits the activity of Na-K-ATPase when measured in a plasma membrane preparation. A preliminary experiment showed that mercury did not inhibit rubidium uptake into separated rectal gland tubules until its concentration reached 0.1 mM. At that concentration it also inhibited ouabain-insensitive uptake, suggesting a non-specific effect . In the present report we extended these experiments and also examined the effect of mercury on the efflux of chloride and potassium.

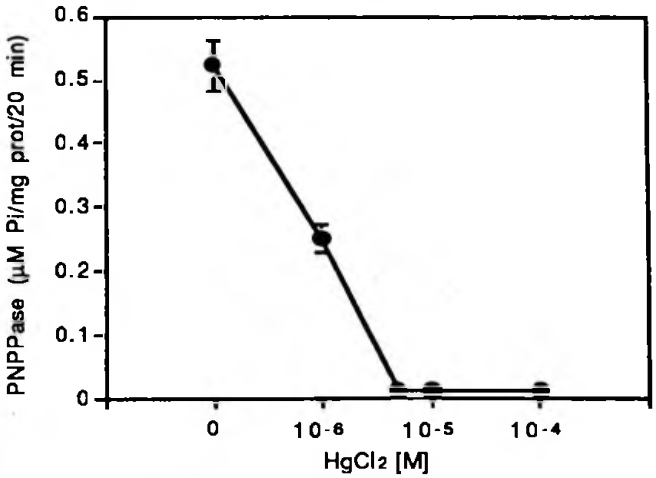

Figure 1. Effect of mercuric chloride on p-nitrophenylphosphatase activity. Half maximal inhibition was found at a concentration of 10^{-6}M and complete inhibition at 5 x 10^{-6}M.

To measure the effect of mercury on Na-K-ATPase we assayed its effect on the phosphatase activity of the enzyme, measured by its capacity to hydrolyze p-nitrophenylphosphate (PNPP), in a plasma membrane preparation of rectal gland. Figure 1 shows that mercury inhibits the PNPPase activity of Na-K-ATPase in a dose dependent way with half maximal inhibition at 10^{-6}M.

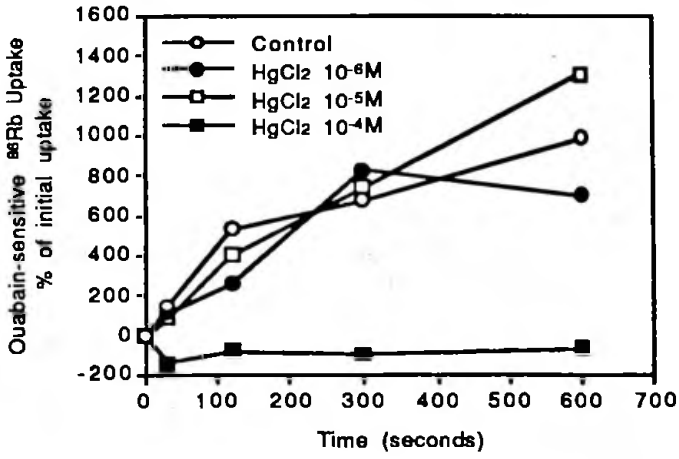

Figure 2. Effect of mercuric chloride on ^{86}Rb uptake into separated rectal gland tubules. Mercuric chloride had no effect on ^{86}Rb uptake at concentration of 10^{-6}M or 10^{-5}M. At 10^{-4}M mercuric chloride inhibited not only ouabain-sensitive but also ouabain-insensitive uptake. Values are the average of six experiments.

We repeated our previous measurement of rubidium uptake into separated rectal gland tubules using the methods previously described (Silva, P. et al. Bull. MDIBL 1994, 33:79). Figure 2 shows that mercuric chloride inhibited the uptake of rubidium only at a concentration of 10^{-4}M. As noted previously, this concentration of mercury also inhibited ouabain insensitive rubidium uptake. Thus, mercury inhibits Na-K-ATPase

in vitro but has little effect on the enzyme in an intact cell preparation. In the isolated perfused rectal gland the concentration of mercuric chloride that produces half-maximal inhibition of chloride secretion is ~10^{-5}M (Silva, P. et al. Comp. Biochem. Physiol. 103C:569, 1992). The discrepancy between the in vitro and in vivo effects of mercury suggests that the previously observed inhibitory effect of mercury on chloride secretion by the perfused rectal gland is not primarily the result of its inhibitory effect on Na-K-ATPase.

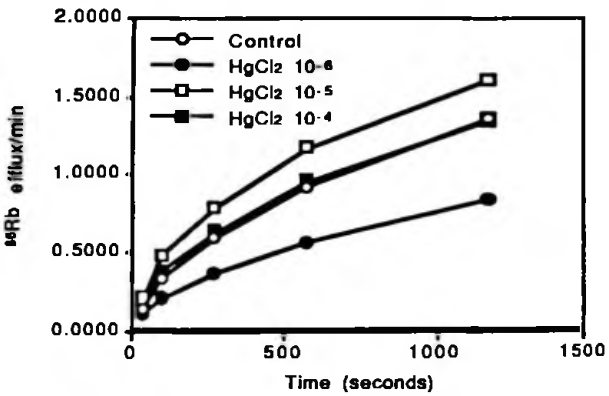

Figure 3. Effect of mercuric chloride on ^{86}R b efflux. Representative experiments showing the efflux of ^{86}Rb from cultured rectal gland cells. Mercuric chloride had no effect on the efflux of ^{86}Rb.

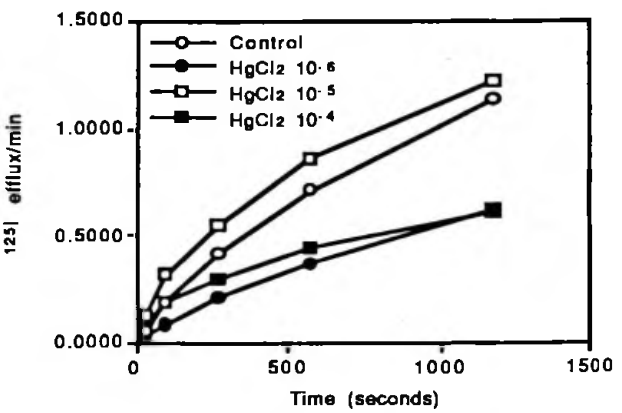

Figure 4. Effect of mercuric chloride on ^{125}I efflux. Representative experiments showing the efflux of ^{125}I from cultured rectal gland cells. Mercuric chloride had no effect on the efflux of ^{125}I.

We next examined the effect of mercury on the efflux of potassium and chloride, two transport sites that participate in the secretion of chloride by the rectal gland. The efflux of chloride and potassium was measured in cultured rectal gland cells using ^{125}I and ^{86}Rb as described by Venglarik et al. (Venglarik, C.J. et al. Am J Physiol. 259:C358-64, 1990). Figures 3 and 4 show the efflux of ^{125}I and ^{86}Rb. Mercury did not alter the efflux of either ^{125}I or ^{86}Rb from cultured rectal gland cells. These experiments suggest that mercury does not inhibit the efflux of chloride or potassium from the rectal gland.

These experiments suggest that the primary inhibitory effect of mercury on the secretion of chloride by the rectal gland is not on Na-K-ATPase, the efflux of chloride or the efflux of potassium. Thus, the inhibitory effect of mercury may be exerted on the 2Cl: Na: K cotransporter that regulates the entry of chloride into the cell.

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