EFFECT OF MERCURIC CHLORIDE ON THE Na-K-2Cl COTRANSPORTER IN THE RECTAL GLAND OF SQUALUS ACANTHIAS: STUDIES WITH ISOLATED PLASMA MEMBRANE VESICLES

Evamaria Kinne-Saffran and Rolf K.H. Kinne
Max-Planck-Institut für molekulare Physiologie,
Abteilung Epithelphysiologie, 44139 Dortmund, FRG

The long term objective of the research is to define at the membrane-molecular level the action of heavy metals on renal transport systems involved in tubular reabsorption and secretion and in the urinary concentrating mechanisms. The thick ascending limb of Henle's loop (TALH) plays a major role in the counter current system and is known to be the site of action of mercurial diuretics.

To investigate further the effects of mercuric chloride on the Na-K-2Cl cotransport system present both in the TALH and in rectal gland, plasma membranes were isolated from dogfish (Squalus acanthias) rectal gland 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. The activity of the Na-K-2Cl cotransporter was determined by measuring the chloride-dependent and/or bumetanide-inhibitable 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].

In Table 1 the effect of preincubating isolated membranes with 0.1 mM $HgCl_2$ on the uptake of rubidium in the presence of a sodium chloride gradient and in the presence of a sodium nitrate gradient or in the presence of 10^{-4} M bumetanide is shown. As demonstrated previously [Hannafin et al., J. Membrane Biol. 75: 73-83, 1993] replacement of chloride by nitrate or the presence of 10^{-4} M bumetanide inhibits Na-K-2Cl cotransport completely. Both fluxes seem to be affected by mercuric chloride. Rubidium uptake in the presence of chloride decreased by an average of 62%, whereas rubidium uptake in the presence of nitrate decreased initially by 30%. Both effects were significant at a $p < 0.05$ level. If the chloride-dependent or bumetanide-inhibitable rubidium uptake is considered, that represents the activity of the Na-K-2Cl cotransporter, mercuric chloride inhibits the transporter in the average by 85%. In the same table the uptake of mannitol by these vesicles is shown. Mannitol enters the vesicles by diffusion and can be used as an indicator for vesicle integrity. No change in mannitol uptake was observed when the membranes had been pretreated with $HgCl_2$, suggesting no disruption of the vesicles. These data indicate that $HgCl_2$ strongly interacts with the Na-K-2Cl cotransport system in the rectal gland. In the mammalian kidney the Na-K-2Cl cotransporter is directly involved in active sodium chloride transport by the thick ascending limb of Henle's loop. This tubular segment is one of the major determinants of renal concentration ability. Since chronic heavy metal exposure is accompanied by a decreased ability to concentrate urine, the thick ascending limb and its transport systems may be the site of damage induced by mercury.

Table 1: Effect of mercuric chloride on Na-K-2Cl cotransport in rectal gland plasma membrane vesicles

	^{86}Rb uptake after 15 s	^{86}Rb uptake after 60 s	^{3}H-mannitol uptake after 15 s	^{3}H-mannitol uptake after 60 s
control	109±12	204±21	9±3	18±5
+ 10^{-4} M bumetanide (or NO_3 gradient)	53.5±7	121±13	9±3	18±5
Na-K-2Cl cotransport	55.5±8	83±10		
+ 1 x 10^{-4} M $HgCl_2$	43.5 (61%)	78 (62%)	9±3	22±4
+ 10^{-4} M bumetanide (or NO_3 gradient)	37 (30%)	56.5 (53%)	9±3	22±6
Na-K-2Cl cotransport	8.5 (85%)	21.5 (83%)		

Values are given in pmoles/mg protein and represent mean values ± SEM of four determinations derived from different membrane preparations. One dogfish was used for each membrane preparation. Number in parentheses indicate the % inhibition observed.

These studies provide the starting point to investigate the site of interaction (sodium binding site, potassium binding site, chloride binding site, bumetanide binding site) of $HgCl_2$ with the Na-K-2Cl cotransporter and to elucidate the chemical species of the inhibitory $HgCl_2$. Future studies will then be devoted to characterize at the protein level those segments of the transport protein whose function is impaired by $HgCl_2$.

Supported by grant NIEHS 1-P30-ES003828