MERSALYL FAILS TO INHIBIT RECTAL GLAND CHLORIDE TRANSPORT IN *SQUALUS ACANTHIAS*: THE ROLE OF CHLORIDE CONCENTRATION

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The shark rectal gland and the thick ascending limb cells of the mammalian nephron both actively transport chloride. In both epithelia, inorganic mercury inhibits epithelial transport. Cells of both epithelia contain a Na-K-2Cl cotransporter which transports Cl ions from the extracellular environment into the cell. Chloride then diffuses out of the cell through Cl channels located on the opposite side of the cell. In the thick ascending limb cells, the Na-K-2Cl cotransporter is located on the apical membrane facing the urinary space. In the rectal gland cells, the Na-K-2Cl cotransporter is located on the basolateral membrane facing the blood. On the other hand, organic mercurials applied to the side of the cell containing the Na-K-2Cl cotransporter inhibit Cl transport in the thick ascending limb cells but not in cells of the rectal gland [Silva et al., Comp. Biochem. Physiol. 1992;103C:569]. The explanation of this difference in sensitivity to organic mercurials is unknown. One difference between these two epithelia lies in the ambient concentration of Na and Cl which is present at the extracellular domain of the Na-K-2Cl cotransporter. Herein we test the hypothesis that organic mercurials compete with Na and/or Cl for binding to the extracellular domain of the Na-K-2Cl cotransporter.

Rectal glands were perfused in vitro as previously described [Solomon et al., Am. J. Physiol. 1992, 262:R707] and ion substitutions of the perfusate were performed. The organic mercurial-inhibitable thick ascending limb usually has a Na and Cl concentration of 20-100 mM at the apical membrane where the Na-K-2Cl cotransporter is located. We therefore prepared shark's Ringers solution [Ibid.] with ion substitutions to achieve comparable Na and Cl concentrations with which to perfuse the basolateral membranes of the rectal gland where the Na-K-2Cl cotransporter is located. Perfusion of the rectal gland at reduced Cl concentrations was accomplished by equimolar substitution of Na gluconate for NaCl. Glands were stimulated to secrete chloride by constant perfusion with theophylline (2.5×10^{-4}M) and cAMP (5×10^{-5}M). Mersalyl was given as a constant infusion (10^{-5}M) during the final 30 minutes of perfusion. This concentration is known to inhibit Cl transport at the thick ascending limb of Henle [Burg and Green, Kidney Int. 1973;4:245]. The concentration of Cl in the perfusate was reduced to either 140 mM or 70 mM without changing Na concentration (280 mM) or total osmolarity. Under these conditions, mersalyl did not inhibit chloride secretion (Figure 1A).

The osmolarity of rectal gland blood is approximately threefold greater than that of urine at the apical membrane of the thick ascending limb cells (1000 vs 300 mosm/kg respectively). To address whether this relative hyperosmolarity also contributed to the lack of effect of mersalyl, we repeated the perfusions in the presence of Na and Cl concentrations of 140 mM and in the absence of urea, substituting 280 mmol mannitol to maintain total perfusate osmolarity at approximately 50% of normal. Under these conditions, mersalyl was also without inhibitory effect (Figure 1B). Attempts to perfuse the shark rectal gland in the absence of mannitol (a perfusate osmolarity of 290 mosm/kg similar to that found in the lumen of the thick ascending limb of Henle) resulted in almost complete cessation of rectal gland secretion. This presumably resulted from osmotic swelling of the cells resulting in inhibition of secretion.

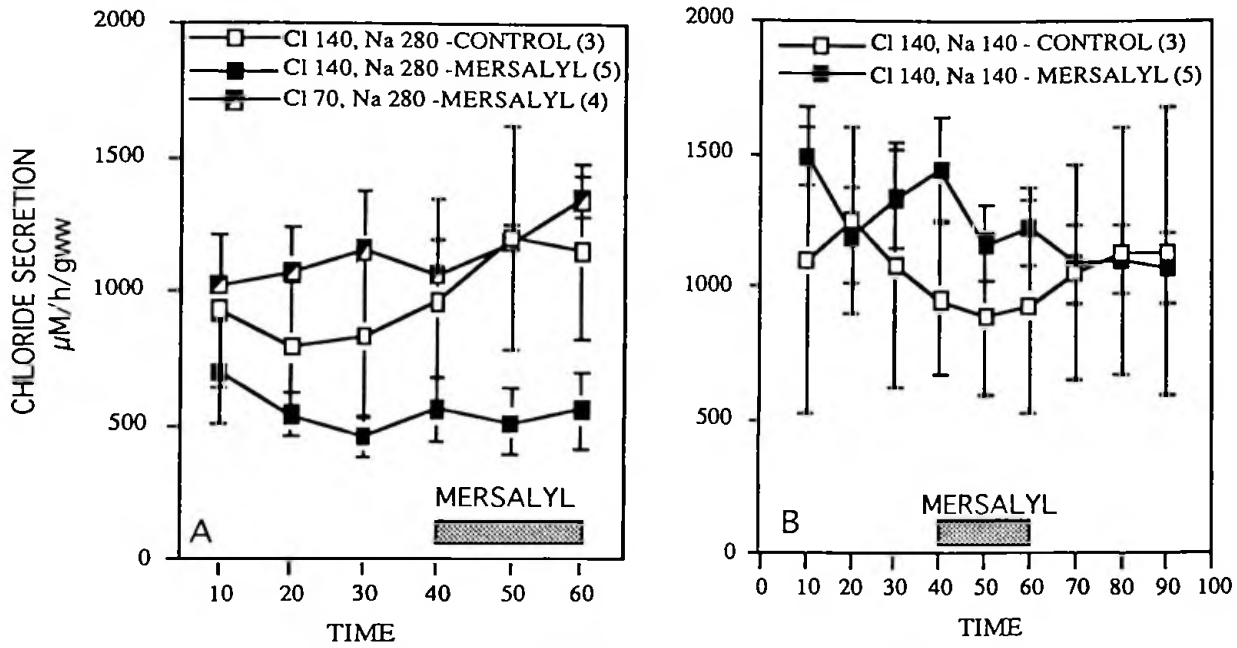

Figure 1: Chloride secretion by the in vitro perfused shark rectal gland under condition of stimulation with theophylline and cAMP. After 30 minutes of perfusion, mersalyl 10^{-5}M was added to the perfusate for a period of 30 minutes (shaded bar). A. Perfusion with 140 or 70 mM Cl, and 280 mM Na (Na gluconate substitution) in the presence of 280 mM urea (total osmolarity 900 mosm/kg). B. Perfusion with 140 mM Na and 140 mM Cl in the presence of 280 mM mannitol without urea (total osmolarity = 600 mosm/kg). In both conditions, mersalyl did not inhibit net chloride secretion by the rectal gland.

These observations are consistent with our previous findings that mersalyl, an organic mercurial, is without an inhibitory effect on transepithelial ion transport in the shark rectal gland. This lack of effect does not appear to be related to the higher ambient sodium and chloride concentration at the extracellular domain of the Na-K-2Cl cotransporter, a putative site of mercurial interaction. These data do not support the hypothesis that the inhibitory effect of organic mercurials on ion transport results from competition with the extracellular binding sites of Na and Cl on the Na-K-2Cl cotransporter.

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