EFFECT OF CLOTRIMAZOLE ON THE SECRETION OF CHLORIDE BY THE SHARK RECTAL GLAND.

Patricio Silva.[1] Christopher Sighinolfi,[2] Katherine Hessler,[3] Richard Hays,[4] Katherine Spokes[5], and Franklin H. Epstein,[5]
[1]Department of Medicine, Temple University Hospital, Philadelphia, PA 19140.
[2]Brewer High School, Brewer, ME 04412
[3]Ellsworth High School, Ellsworth, ME 04421.
[4]Department of Medicine, Albert Einstein College of Medicine, Bronx, NY 10461
[5]Department of Medicine, Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, MA 02215.

The rectal gland of *Squalus acanthias* secretes chloride by a process that has been termed secondary active transport. Chloride enters the cell via the sodium, potassium two chloride cotransporter following the electrochemical gradient for sodium that is directed into the cell. Chloride exits the cell via the shark equivalent of CFTR. Sodium is pumped out of the cell by sodium, potassium ATPase thus maintaining the gradient. Potassium recirculates across the basolateral membrane through one or more potassium channels. In these experiments we used clotrimazole, a specific inhibitor of the Gardos calcium dependent potassium channel, to determine whether the Gardos channel mediates the recirculation of potassium in the rectal gland.

Isolated rectal glands of *Squalus acanthias* were perfused with oxygenated shark Ringer's solution at pH 7.6 as previously described (Silva, P. et al. Methods Enzymol 192:754-66, 1990). Duct fluid was collected at 10 minute intervals in small tared plastic centrifuge tubes and the volume measured every 10 minutes by weighing. The concentration of chloride in the duct fluid was estimated by amperometric titration. An initial thirty minutes of control perfusion (three collection periods) allowed the gland to reach a stable basal state. At the end of this control period a 1 ml bolus of VIP, calculated to deliver a concentration of 10^{-7}M to the gland, was injected directly into the arterial catheter over 1 min. After three ten minute periods, the perfusion solution was changed to one containing clotrimazole at a concentration of 1 or 10 μM. After an additional 30 minutes of washout, another identical bolus of VIP was injected into the rectal gland artery and duct secretion was collected for 3 final ten minute periods. It was thus possible to estimate the effect of VIP on the rectal gland secretion with and without clotrimazole.

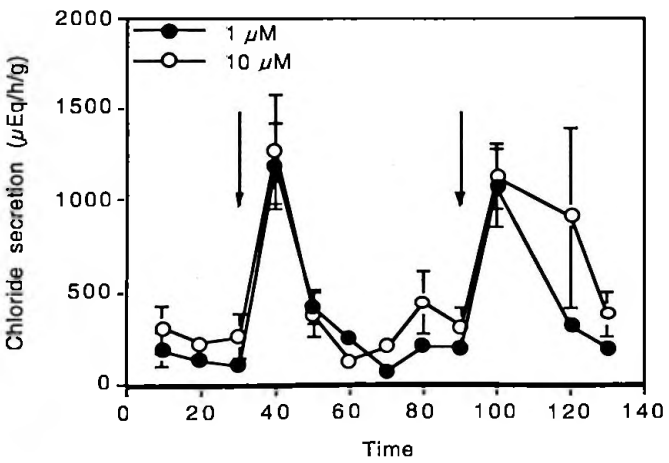

Figure 1. Effect of clotrimazole on stimulation of perfused glands by VIP 10^{-7}M. VIP was injected into the rectal gland artery at the time indicated by the arrows. Clotrimazole had no effect on the stimulation of chloride secretion by VIP. Closed symbols are 1 μM clotrimazole and open symbols are 10 μM. Values are mean±SEM.

The results are summarized in Figure 1. Perfusion with clotrimazole did not

inhibit the stimulation of the secretion of chloride induced by VIP. The maximum rate of secretion after VIP was the same in the presence or absence of clotrimazole. The recirculation of potassium, necessary for the secretion of chloride, is not inhibited by blockade of the Gardos channel. The rectal gland may not have a Gardos channel or alternatively, there may be additional potassium channels that can be recruited for the extrusion of potassium.

Supported in part by grant NIH 5 P30 ES03828-16 and NSF DBI-0139190