# INHIBITORS OF PHOSPHATASES DO NOT ALTER CHLORIDE SECRETION BY THE RECTAL GLAND OF *SQUALUS ACANTHIAS*.

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Cellular phosphatases terminate the activity of phosphorylated proteins by removing phosphate from the phosphorylated sites. Inhibition of cellular phosphatases is then expected to mimic the effect of agonist kinases or to enhance their action. A recent report indicates that inhibition of PP-2B stimulates chloride transport in 3T3 cells expressing CFTR (Fischer, H. et al. *Pflugers Arch* 1998, 436:175-81). Similarly, inhibition of phosphatases PP-1 and PP-2A increase steady state current in the opercular epithelium of the killifish, a chloride transporting tissue (Hoffmann, E. et al. *The Bulletin Mt. Desert Island Bio. Lab.* 37:70-2, 1998). The present experiments were designed to examine the role of cellular phosphatases on chloride transport in the isolated perfused rectal gland of the spiny dogfish, *Squalus acanthias*.

Shark rectal glands were perfused as described in Silva P, et al. *Methods Enzymol.* Vol 192:754-66, 1990. Cyclosporine A (CsA) and FK-506 were used to inhibit phosphatase PP-2B and okadaic acid to inhibit PP-1 and PP-2A. There was an initial thirty minute control period (three collection periods) to allow the secretion of chloride to reach a stable rate. At the end of this control period, 4 x $10^{-6}$M forskolin, final concentration, was injected as a bolus into the arterial catheter over a period of one minute and collections continued for an additional thirty minutes at the end of which time chloride secretion had returned to basal rates. The perfusate was then changed to one containing a phosphatase inhibitor for either thirty minutes or one hour and the bolus of forskolin repeated. Control experiments were done without the phosphatase inhibitor.

CsA at a concentration of $10^{-5}$M infused for thirty minutes produced no effect on chloride secretion. CsA did not enhance or otherwise change the stimulation of chloride secretion induced by forskolin. Figures 1 and 2 summarize the results. Forskolin evoked the same rate of secretion of chloride in the presence and absence of CsA. CsA at a concentration of $10^{-6}$M penetrates the rectal gland cells and distributes throughout the cellular cytoplasm as determined by immunocytochemistry (G. Fricker, personal communication).

To test further the role of phosphatase PP-2B on chloride transport we used FK-506, another PP-2B inhibitor with higher affinity for PP-2B than CsA. As was the case with CsA, FK-506 neither enhanced nor changed the effect of forskolin, nor did it have a stimulatory effect of its own. Figure 3 summarizes the results.

To examine the role of PP-1 and PP-2A we used okadaic acid, which inhibits both phosphatases. Okadaic acid neither stimulated chloride secretion nor enhanced the stimulatory effect of forskolin on chloride secretion. Figure 4 summarizes the results. In a separate series of experiments okadaic acid failed to alter the stimulatory effect of CNP.

Figure 1. The stimulatory effect of a bolus of 4 x $10^{-6}$M forskolin (arrows) was not altered by a thirty minute infusion of $10^{-5}$M cyclosporine. Cyclosporine did not change the basal rate of secretion of chloride. Values are mean±SEM, n=6 for glands perfused with cyclosporine (closed circles) and 6 for those perfused without cyclosporine (open circles).

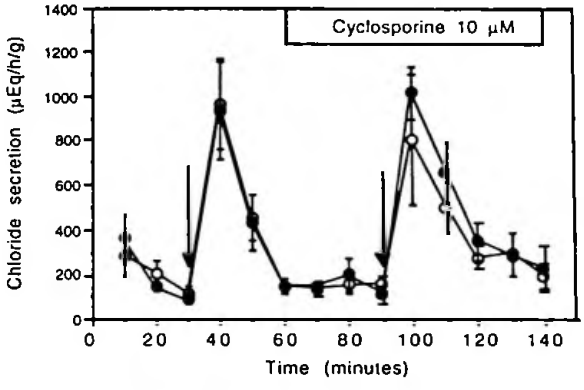



Figure 2. The stimulatory effect of a bolus of 4 x $10^{-6}$M forskolin (arrows) was not changed by perfusing with $10^{-6}$M cyclosporine for sixty minutes. Again, cyclosporine by itself did not change the rate of secretion of chloride. Values are mean±SEM, n=6 for glands perfused with cyclosporine (closed circles) and 6 for those perfused without cyclosporine (open circles).

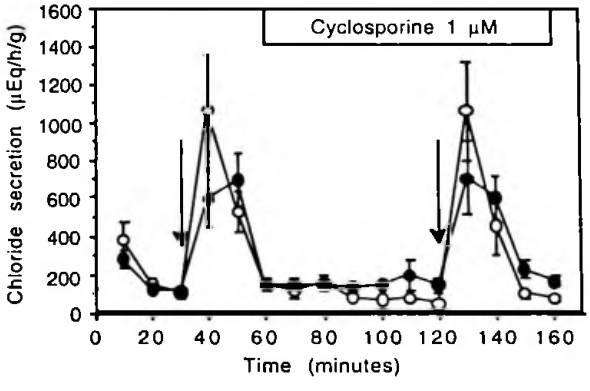



Figure 3. FK-506, $10^{-7}$M had no effect on the stimulatory effect of a bolus of 4 x $10^{-6}$M forskolin (arrows). FK-506 was infused for sixty minutes. FK-506 alone had no effect on the rate of secretion of chloride. Values are mean±SEM, n=6 for glands perfused with FK-506 (closed circles) and 6 for those perfused without FK-506 (open circles).

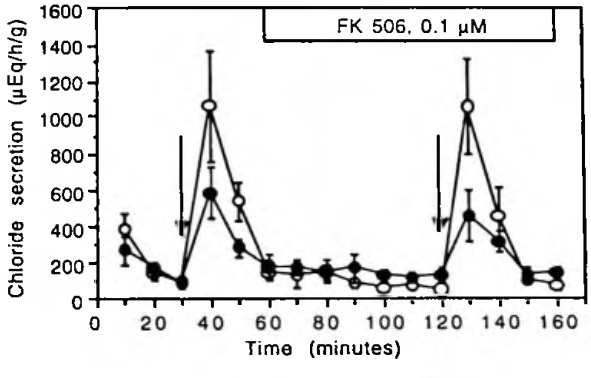
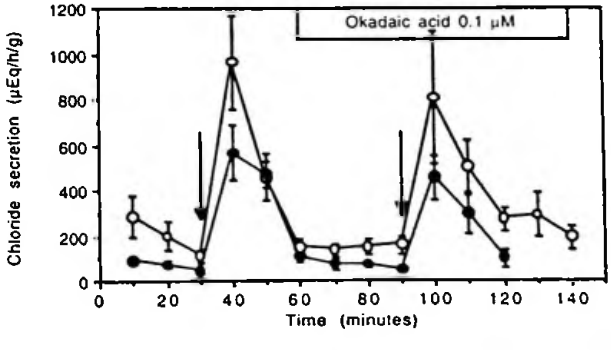



Figure 4. Okadaic acid, $10^{-6}$M, had no effect on the stimulatory effect of 4 x $10^{-6}$M forskolin (arrows). Okadaic acid was infused for sixty minutes. The stimulatory effect of forskolin was the same in the presence or absence of okadaic acid. Okadaic acid alone had no effect on the rate of secretion of chloride. Values are mean±SEM, n=5 for glands perfused with okadaic acid (closed circles) and 6 for those perfused without it (open circles).



The observations that cyclosporine and FK-506 do not enhance chloride secretion by the perfused gland suggest that dephosphorylation mediated by PP-2B does not play a major role in the inactivation of the chloride secretory pathway. The failure of cyclosporine and FK-506 to enhance the effect of forskolin provides further support to the notion that PP-2B is not critically involved in the process of activation/inactivation of the secretion of chloride. Similarly, the failure of okadaic acid either to stimulate the secretion of chloride or to alter the stimulatory effects of forskolin and CNP suggest that phosphatases PP-1 and PP-2A do not provide the major process of inactivation of the secretion of chloride by the rectal gland of the shark.

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