# ROLE OF CYTOSKELETON IN THE SECRETION OF CHLORIDE IN THE RECTAL GLAND OF SQUALUS ACANTHIAS.

Patricio Silva,[1] Nina Derby,[2] Martin Schnermann,[3] Katherine Spokes[4], and Franklin H. Epstein.[4]
Departments of Medicine, [1]Temple University Hospital, Philadelphia, PA 19140, and [4]Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, MA 02215. [2]Smith College, Amherst, MA 02215. [3]Colby College, Waterville, ME 04901.

Actin and actin-associated proteins such as myosin light chains are involved in the transport of chloride and the secretion of hormones. We have previously shown that inhibition of myosin light chain kinase (MLCK) with ML-7, a specific inhibitor of MLCK, inhibits the effect of C-type natriuretic peptide to stimulate chloride secretion by the rectal gland of the spiny dogfish, Squalus acanthias. In those experiments, ML-7 did not inhibit the effect of forskolin, which directly activates adenylate cyclase, to stimulate the secretion of chloride. In the present series of experiments we examined the role of MLCK and of actin in the mechanism of chloride secretion stimulated by CNP (C-type natriuretic peptide), VIP (vasoactive intestinal peptide), and by forskolin.

Shark rectal glands were perfused as described in Silva, P. et al. (Methods Enzymol 192:754-66, 1990). The secretion of chloride was stimulated with either VIP, CNP, or forskolin, given as a bolus infusion over a period of 1 minute at the dose calculated to expose the perfused gland to $10^{-8}$M for VIP, and $5x10^{-7}$M for CNP, and $10^{-5}$M for forskolin. In the experiments with CNP, the glands were perfused with or without procaine, $10^{-2}$M. ML-7 was used at a concentration of $3x10^{-6}$M. Cytochalasin D, an alkaloid that binds to the barbed end of actin filaments and prevents their elongation while allowing their degradation, was used at a concentration of $10^{-6}$M. Both ML-7 and cytochalasin D were perfused throughout the experiments in which they were used. The secretion of chloride was collected for consecutive 10 minute periods. 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, boluses of either VIP or CNP were added to the perfusate and collections continued for an additional thirty minutes.

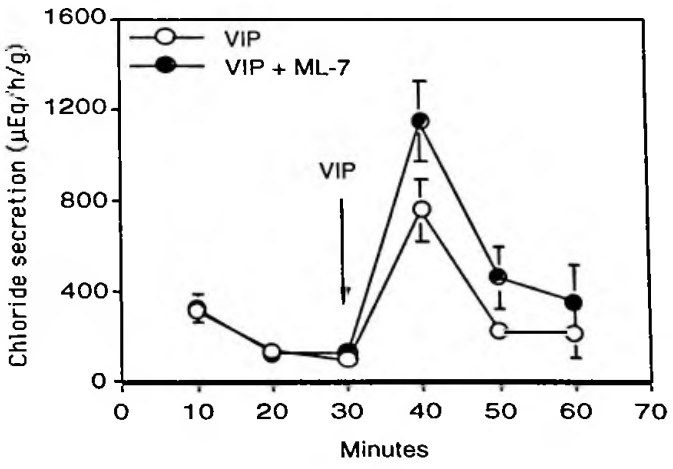



Figure 1. VIP 10-8M significantly stimulated the secretion of chloride in both the control perfusions and also in those perfused with 3 x $10^{-6}$M ML-7 ($p < 0.01$ for both sets of experiments). VIP was added at the time indicated by the arrow. The rate of chloride secretion achieved with VIP in the presence of ML-7 was significantly higher than that without ML-7 ($\ddot{p} = 0.005$). Values are mean±SEM, n=18 for glands perfused without ML-7 and n=7 for those perfused with ML-7. All comparisons were done using Anova for repeated measures.

Neither ML-7 (Figure 1) nor cytochalasin (Figure 2) prevented the stimulatory effect of VIP. VIP stimulated the secretion of chloride both in the presence and absence of ML-7 or cytochalasin D.

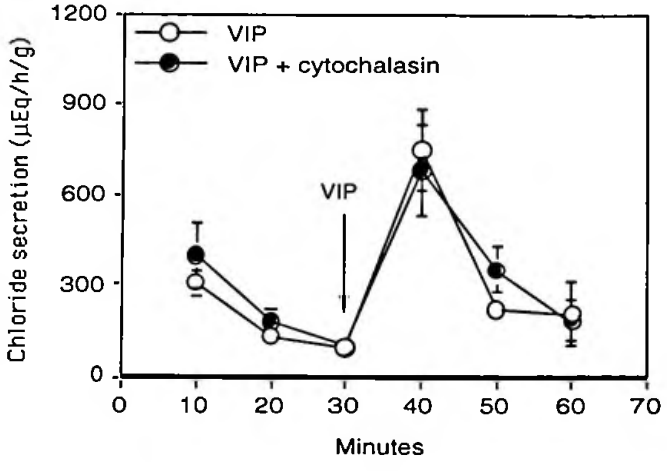



Figure 2. Cytochalasin D did not prevent the stimulation of the secretion of chloride by VIP. VIP was added at the time indicated by the arrow. VIP significantly increased the secretion of chloride in glands perfused with or without cytochalasin D ($p < 0.01$ for both sets of experiments). There was no difference in the rate of chloride secretion in those perfused with or without cytochalasin D. Values are mean±SEM, n=6 for glands perfused with cytochalasin D and n= I8 in those without cytochalasin D. All comparisons were done using Anova for repeated measures.

Both ML-7 (Figure 3) and cytochalasin D (Figure 4) reduced the stimulatory effect of CNP $5x10^{-7}M$ on chloride secretion. In a separate series of experiments glands were perfused with procaine, $10^{-2}M$, to prevent the release of VIP during perfusion with CNP. In these perfusions, cytochalasin D completely prevented the stimulatory effect of CNP.

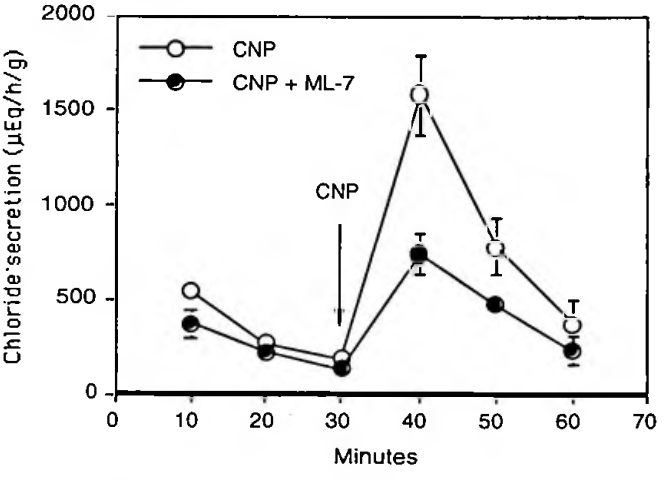



Figure 3. ML-7 reduces the stimulation of the secretion of chloride by CNP. CNP was added at the time indicated by the arrow. CNP significantly increased the secretion of chloride in glands perfused without ML-7 ($p < 0.01$). There was a significant reduction in the rate of chloride secretion stimulated by CNP in those perfused with ML-7 ($p = 0.009$). Values are mean±SEM, n=7 for glands perfused with ML-7 and without ML-7. All comparisons were done using Anova for repeated measures.

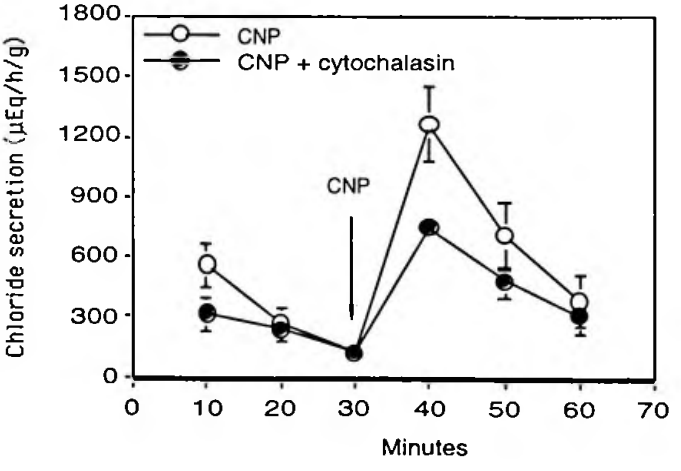
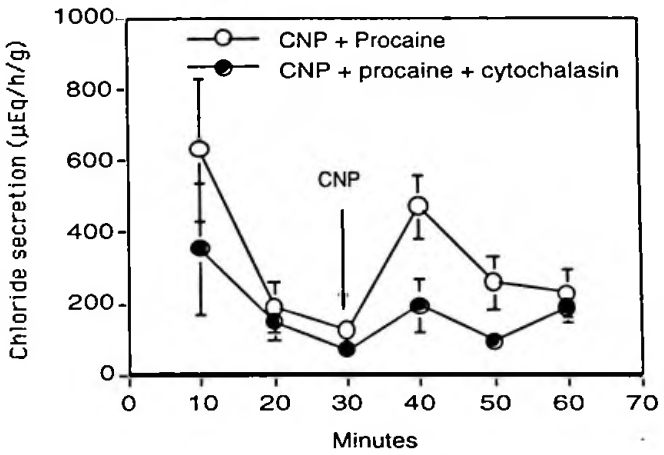


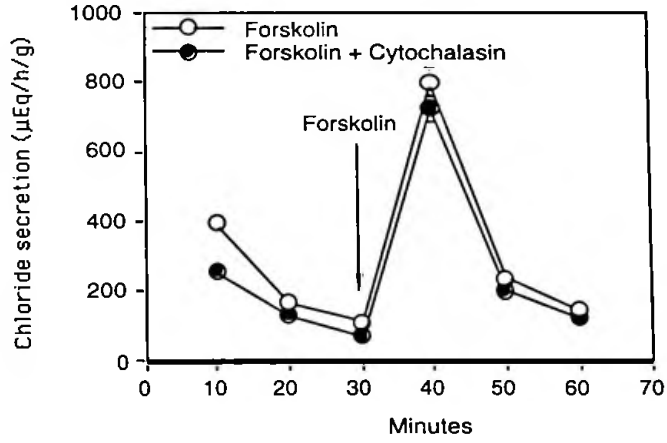

Figure 4. Cytochalasin D reduces the stimulation of the secretion of chloride by CNP. CNP was added at the time indicated by the arrow. CNP stimulated the secretion of chloride in the glands perfused without cytochalasin D ($p < 0.01$). Cytochalasin D significantly reduced the stimulation of chloride secretion evoked by CNP ($p = 0.022$). Values are mean±SEM, n=6 for glands perfused with and without cytochalasin D. All comparisons were done using Anova for repeated measures.

Figure 5. Cytochalasin D prevents the stimulation of the secretion of chloride by CNP in glands perfused with procaine. CNP was added at the time indicated by the arrow. CNP significantly increased the secretion of chloride in glands perfused without cytochalasin D ($p < 0.01$). There was no significant increase in the rate of chloride secretion in those perfused with cytochalasin D ($p > 0.05$). The secretion of chloride in the glands receiving cytochalasin was significantly different from those without the alkaloid ($p = 0.024$). Values are mean±SEM, n=9 for glands perfused with and without cytochalasin D. All comparisons were done using Anova for repeated measures.




Cytochalasin D did not significantly reduce the stimulatory effect of forskolin given as a one minute bolus (final concentration $10^{-5}$M)(Figure 6).

Figure 6. Cytochalasin D does not prevent the stimulation of the secretion of chloride by forskolin. Forskolin $10^{-5}$M was added at the time indicated by the arrow. Forskolin significantly increased the secretion of chloride in glands perfused with and without cytochalasin D ($p < 0.01$). Values are mean±SEM, n=10 for glands perfused without cytochalasin D and n=4 for those perfused with cytochalasin. All comparisons were done using Anova for repeated measures.




The stimulatory effect of VIP and of forskolin is not inhibited by ML-7 or cytochalasin D, and is therefore unlikely to involve either MLCK or actin microfilaments. On the other hand, the direct effect of CNP to stimulate rectal gland cells (measured in the presence of procaine) is almost completely blocked by cytochalasin D, as it was by ML-7. These results suggest that MLCK and the integrity of actin filaments are necessary for the direct effect of CNP on chloride secretion by the rectal gland. We have previously shown that the direct effect of CNP is also blocked by staurosporine, suggesting that CNP stimulation is mediated by protein kinase C. These present results also suggest that the secretion of chloride by the rectal gland is mediated by at least two different intracellular pathways, one activated by VIP and mediated by cAMP and PKA, and another activated by CNP and mediated in part by the cytoskeleton.

Supported in part by a summer research fellowship of The American Heart Association to Nina Derby, a grant from the National Science Foundation to MDIBL (DBI-9820400) for Research Experiences in Marine Molecular Physiology (Martin Schnermann), and a gift from David and Edith Milbury.