ML-7 INHIBITS CNP-STIMULATED BUT NOT FORSKOLIN-STIMULATED CHLORIDE SECRETION IN THE RECTAL GLAND OF *SQUALUS ACANTHIAS.*

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Myosin light chain kinase (MLCK) is involved in the process of cellular secretion, including that of many different hormones. MLCK can be specifically inhibited by ML-7, a diazepine derivative. In the present series of experiments we examined the effect of ML-7 on chloride secretion by the rectal gland of the dogfish, *Squalus acanthias*.

Shark rectal glands were perfused as described in Silva, P. et al. (*Methods Enzymol.* Vol 192:754-66, 1990). The glands were perfused with or without ML-7, 5 x 10^{-6}M and with or without procaine, 10^{-2}M. 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, 5 x 10^{-7}M CNP, or 4 x 10^{-6}M forskolin, final concentration, were added to the perfusate and collections continued for an additional thirty minutes.

ML-7 did not prevent the stimulatory effect of forskolin, Figure 1 summarizes the results. Forskolin stimulated the secretion of chloride both in the presence and absence of ML-7.

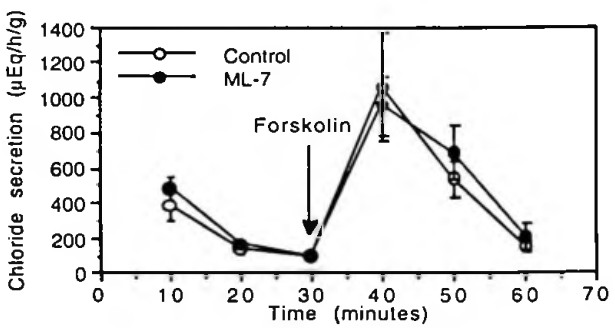

Figure 1. Forskolin significantly stimulated the secretion of chloride in both the control perfusions and also in those perfused with ML-7 ($p < 0.01$ for both sets of experiments). There was no significant difference in the rate of chloride secretion achieved with forskolin between the glands perfused with ML-7 and those without it. Values are mean±SEM, n=6 for glands perfused with and without ML-7.

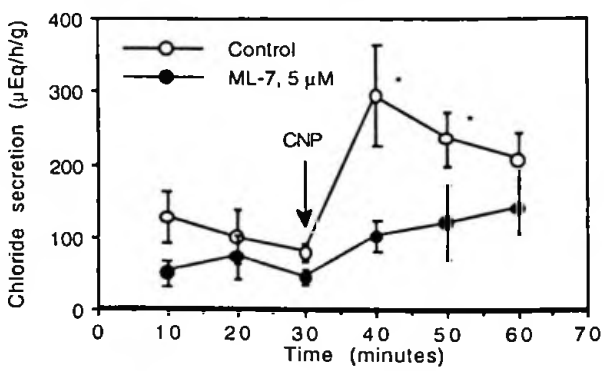

Figure 2. ML-7 prevents the stimulation of the secretion of chloride by CNP in glands perfused with procaine. CNP significantly increased the secretion of chloride in glands perfused without ML-7 ($p < 0.01$). There was no significant increase in the rate of chloride secretion in those perfused with ML-7. Values are mean±SEM, n=6 for glands perfused with ML-7 and n=11 in those without ML-7.

In a separate series of experiments glands were perfused with procaine 10^{-2}M to prevent the release of neurotransmitters during perfusion with CNP. In these perfusions, ML-7 prevented the stimulatory effect of CNP. We conclude from these experiments that the effect of forskolin is not inhibited by ML-7 and therefore unlikely to be mediated by the activation of MLCK. On the other hand, the effect of CNP is almost completely blocked by ML-7. These results suggest that MLCK mediates the direct effect of CNP but not that of forskolin on chloride secretion by the rectal gland. These results also suggest that the secretion of cloride in the rectal gland is mediated by at least two different intracellular pathways, one mediated by cAMP and PKA, and another mediated by MLCK. We have previously shown that staurosporine blocks the effect of CNP to stimulate the secretion of chloride. In those experiments we used staurosporine at a concentration of 10^{-8}M, a concentration that is 8 times greater than the IC_{50} of staurosporine on MLCK. It is therefore possible that in those experiments staurosporine was blocking not only protein kinase C but also MLCK.

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