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

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Previous work from our laboratory has shown that the actin cytoskeleton is involved in the C-type natriuretic peptide-induced stimulation of the transport of chloride by the rectal gland of Squalus acanthias, while the stimulation induced by VIP initiated cascade is independent of the cytoskeleton (Silva, P. et al. Bull MDIBL 39:5-7, 2000). In those experiments we used ML-7 to inhibit myosin light chain kinase, an actin associated kinase, and cytochalasin D, which binds to the barbed end of the actin filaments and prevents their extension. In the present experiments we examined the effect of a different inhibitor of the actin microfilaments, jasplakinolide, a cyclodepsipeptide produced by Jaspis johnstoni, that stabilizes the actin filaments and reorganizes them into tight layer along the plasma membrane of the cell (Posey, SC et al. J Biol Chem 274:4259-65, 1999).

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 or CNP, given as a bolus infusion over a period of 1 minute at the dose calculated to expose the perfused gland to 5 or 7 x 10^{-9}M for VIP, and 5x10^{-7}M for CNP. In all experiments the glands were perfused with procaine 10^{-2}M to prevent the release of VIP from nerves within the rectal gland. Jasplakinolide was used at a concentration of 2 x 10^{-7}M. After a control period of perfusion consisting of three 10-min collections, during which a stable basal secretory rate was established, a bolus of 5 or 7 x 10^{-9}M VIP (V-3628, Sigma Chemical Co.) or 5 x 10^{-7}M C-type natriuretic peptide (N-8768, Sigma Chemical Co.) was given over 1 min, without altering the rate of gland perfusion. Collections were continued at 1-min intervals until the rate of secretion returned to baseline levels. Jasplakinolide (2 x 10^{-7} M) in oxygenated shark Ringer's solution was then infused for the ensuing 40 min. Twenty minutes after the beginning of the jasplakinolide infusion another bolus of VIP or CNP was injected, and 20 minutes later the infusion of jasplakinolide was stopped and replaced by shark Ringer's solution. After the secretory rate had again stabilized, a third 1-minute bolus of the stimulating agent was given, and the effect again observed during successive 1-min clearance periods.

Jasplakinolide significantly reduced the stimulatory effect of CNP 5x10^{-7}M on chloride secretion. The effect of jasplakinolide was reversible. The response of the secretion of chloride to CNP returned to levels not significantly different from those of the initial control after removal of the jasplakinolide (Figure 1).

In a separate series of experiments glands were stimulated with VIP 5 or 7 x 10^{-9}M. In these experiments the response to the first dose of VIP was greater than to the following two doses, as depicted in the control experiments. Jasplakinolide had no effect on the secretion of chloride stimulated by VIP. The pattern of response to VIP in the experiments where jasplakinolide was used was the same as that of the control experiments (Figure 2).

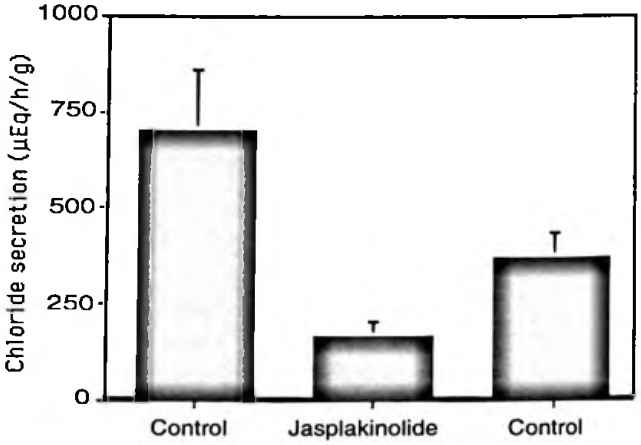

Figure 1. Jasplakinolide reduces the stimulation of the secretion of chloride by CNP. The columns represent the peak rate of secretion of chloride after the infusion of a bolus of 5 x 10^{-7}M CNP, under control conditions, during perfusion with jasplakinolide, and after removal of jasplakinolide. Each gland served as its own control. The graph represents the average of 7 experiments. Jasplakinolide almost completely suppressed the stimulatory effect of CNP, $p<0.001$.

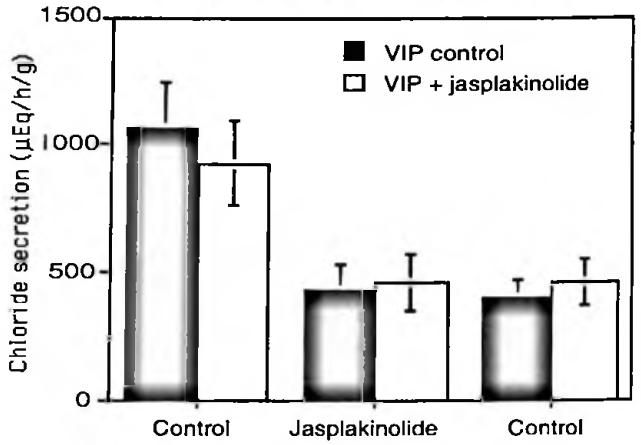

Figure 2. Jasplakinolide had no effect on the stimulation of chloride secretion by VIP. The open bars represent experiments in which jasplakinolide was infused for forty minutes strating ten minutes prior to the second bolus of VIP and continuing for another thirty minutes. The closed bars represent glands that received three consecutive boluses of VIP without jasplakinolide. Each gland served as its own control. The response to the initial dose of VIP was greater than to the second and third doses with or without jasplakinolide. Jasplakinolide had no effect on the stimulatory effect of VIP. The graph represents the average of 6 experiments with and without jasplakinolide.

The stimulatory effect of VIP is not inhibited by jasplakinolide, or, as previously shown, by ML-7 or cytochalasin D, suggesting that the VIP-initiated stimulation of chloride transport does not involve the actin cytoskeleton. 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 jasplakinolide as it was by cytochalasin D, and ML-7. These results confirm our previous finding and suggest that the integrity of actin filaments is necessary for the direct effect of CNP on chloride secretion by the rectal gland. We have previously shown that the stimulatory effects of CNP and VIP differ in their intracellular mediators. The direct effect of CNP is blocked by staurosporine while that of VIP is not, suggesting that CNP stimulation is mediated by protein kinase C. We have also shown that stimulation of the rectal gland via CNP takes appreciably longer than that via VIP suggesting that these modes of stimulation have different pathways of intracellular second-messenger activation. These and the present results 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.

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