STAUROSPORINE AND CYTOCHALASIN D DO NOT INHIBIT THE CHLORIDE SECRETORY RESPONSE TO CONSTANT INFUSIONS OF SHARK C-TYPE NATRIURETIC PEPTIDE

Suzanne J. Forrest[1], Andrea Pelletier[1], Sarah Decker[2], John N. Forrest Jr. [1]
[1]Dept. of Medicine, Yale University School of Medicine, New Haven, CT 06510
[2] Middlebury College, Middlebury VT, 05753

Silva et al. have proposed that the action of C-type natriuretic peptide in the shark rectal gland may involve protein kinase C and actin filaments because both staurosporin, an inhibitor of protein kinase C, and cytochalasin D, an inhibitor of actin filaments, blunt the response to boluses of CNP (Silva et al., Am. J. Physiol. 277: R1725-R1732, 1999; Silva et al., Bull MDIBL 39: 5-7, 2000). Because bolus injections result in variable and uncertain concentrations of these agents, we carried out experiments with staurosporine and cytochalasin D using constant infusions of secretagogues. Additionally, we performed blinded paired experiments (to control for factors such as temperature and observer bias) and measured chloride secretion rates at one minute intervals to provide greater sensitivity to small changes in secretion.

Staurosporine (10 nM) did not inhibit the chloride secretory response to 1.5 nM VIP (figure 1) or 3 nM CNP perfused at constant concentrations (figure 2). Similarly, cytochalasin D did not inhibit the chloride secretory response to a constant infusion of shark CNP (figure 3).

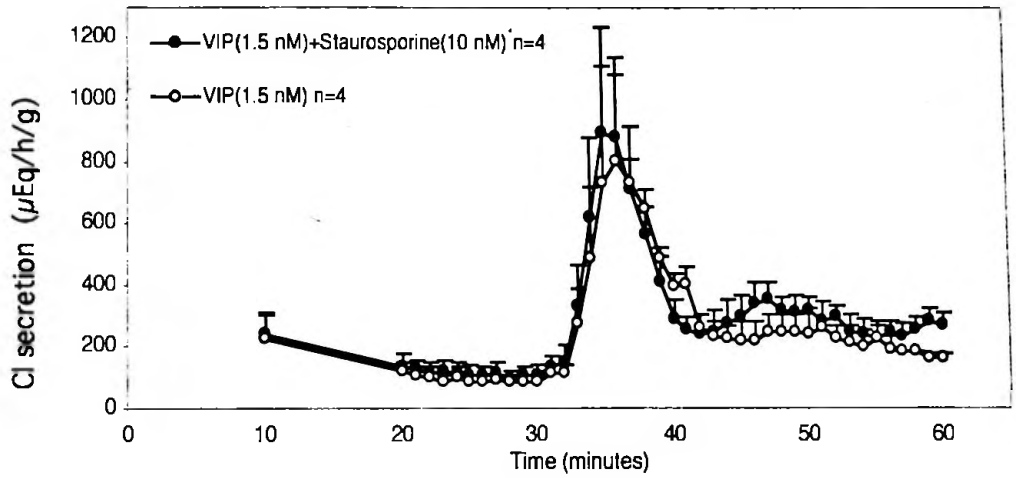

Figure 1. Chloride secretory response to 1.5 nM VIP added at time=30 in the presence and absence of staurosporine (10nM) added at time=0. All perfusate also contained BSA 0.1mg/ml.

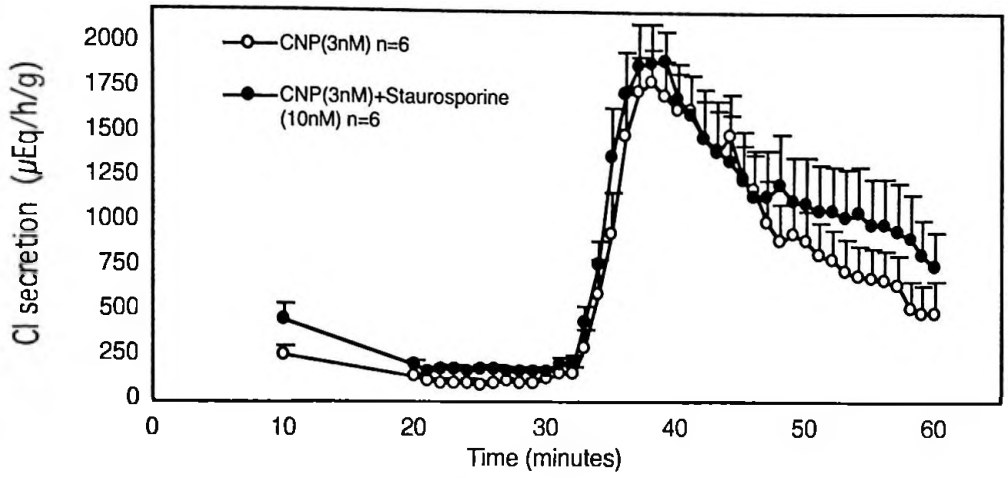

Figure 2. Chloride secretory response to 3 nM sCNP added at time=30 in the presence and absence of staurosporine (10nM) added at time=0. All perfusate also contained BSA 0.1mg/ml.

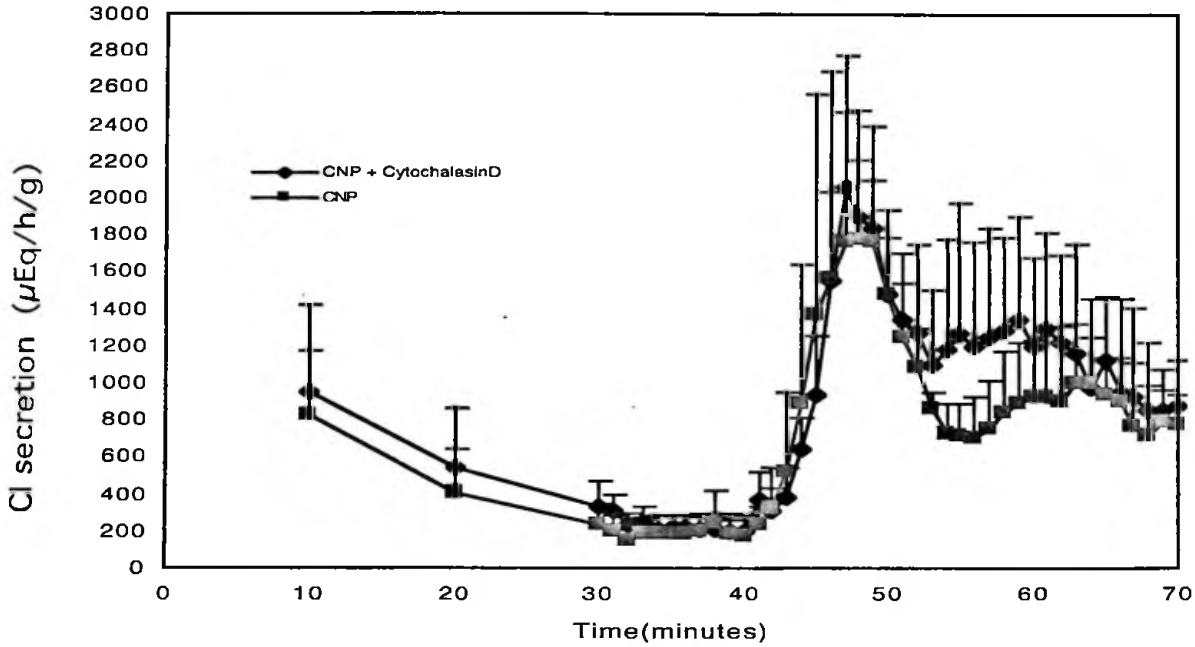

Figure 3. Chloride secretory response to 3 nM CNP added at time=30 in the presence and absence of cytochalasin D (1μM) added at time=0. All perfusate also contained BSA 0.1mg/ml. n=3 paired experiments.

In summary, using paired blinded experiments, one minute measurements and constant infusion of secretagogues and inhibitors, we did not observe effects of either staurosporine or cytochalasin D on CNP stimulated chloride secretion in the perfused gland. These studies raise the possibility that the action of CNP may not involve protein kinase C or actin filaments. Other techniques, such as antisense morpholino oligomers, may be necessary to resolve these possible protein-protein interactions.

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