COLCHICINE DOES NOT PREVENT STIMULATION OF SHARK RECTAL GLAND BY VIP OR CNP.

Patricio Silva,[1] Christopher Signolfi,[2] Jana Richards,[3] Richard Hays,[4] Katherine Spokes,[5] and Franklin H. Epstein.[5]

Departments of Medicine, [1]Temple University Hospital, Philadelphia, PA 19140,
[4]Albert Einstein College of Medicine, Bronx, NY 10461,
[5]Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, MA 02215.
[2]Brewer High School, Brewer, ME 04412.
[3]Bucksport High School, Bucksport, ME 04421.

Previous work from our laboratory has suggested that the actin cytoskeleton is involved in the stimulation of chloride secretion induced by C-type natriuretic peptide (CNP) in the rectal gland of *Squalus acanthias*, while the stimulation induced by vasoactive intestinal peptide (VIP) is independent of it (Silva, P. et al. Bull MDIBL 39:5-7, 2000 and Bull MDIBL 40:27-8, 2001), In the present experiments we examined the role of the microtubular component of the cytoskeleton using colchicine to disrupt the intracellular microtubules.

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 one minute at the dose calculated to expose the perfused gland to 10^{-7}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. Colchicine was used at a concentration of 2.5×10^{-5}M or 10^{-4}M throughout the experiment. After a control period of perfusion consisting of three 10-min collections, during which a stable basal secretory rate was established, a bolus of 10^{-7}M VIP (Sigma Chemical Co.) or 5×10^{-7}M C-type natriuretic peptide (Sigma Chemical Co.) was given over one min, without altering the rate of gland perfusion. Collections were continued at ten minute intervals. Chloride in the rectal gland secretion was measured by amperometric titration using a Buchler-Cotlove chloridometer.

The results are summarized in Figures 1 and 2. Perfusion with 25 μM or 100 μM colchicine did not inhibit the stimulation of the perfused rectal glands by CNP or by VIP.

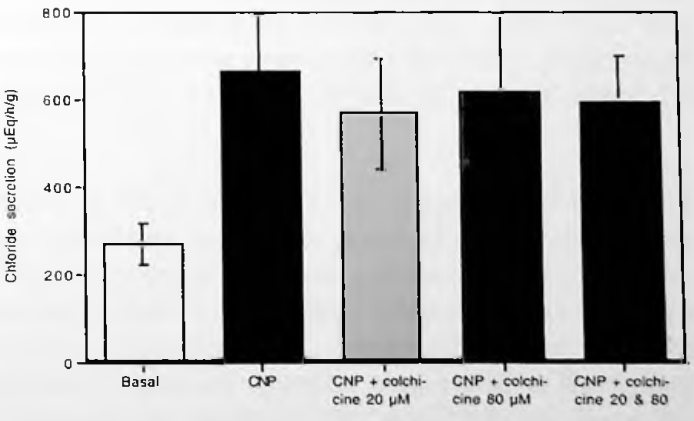

Figure 1. Effect of colchicine on stimulation of perfused glands by CNP. Columns represent the average of 3 ten minute collection periods ± standard error of the mean. CNP was given over one minute as a bolus at a final concentration of 5×10^{-7}M. n=5 for Basal and CNP, n=8 for colchicine 20 μM, n=4 for colchicine 80 μM, and n=12 for both colchicine doses combined. Colchicine did not inhibit stimulation by CNP when present in the perfusion at a concentration of 20 or 80 μM.

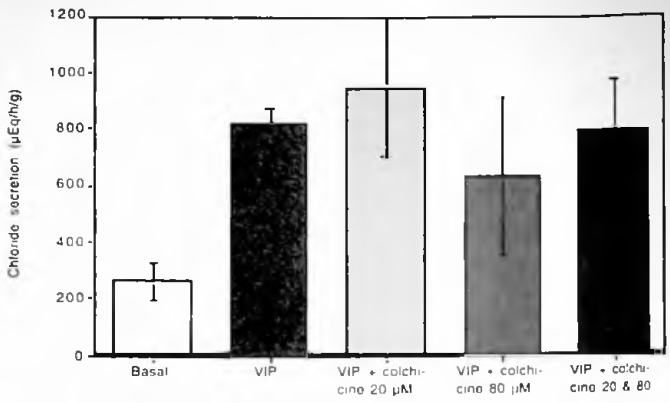

Figure 2. Effect of colchicine on stimulation of perfused glands by VIP. Columns represent the mean ± standard error of the mean of three consecutive 10 minute periods before (Basal) and after the infusion over 1 minute of 10^{-7}M VIP (final concentration). Colchicine 20 or 80 µM did not inhibit stimulation by VIP. n=6 for basal and VIP, n=6 for 20 µM colchicine, n=4 for 80 µM colchicine, and n=10 for 20 and 80 µM colchicine.

These results suggest that, as contrasted with nocodazole, colchicine at the doses used in these experiments does not inhibit the secretion of chloride stimulated by CNP or VIP.

Supported in part by MDIBL and NIEHS P30 ESO3828-16.