EFFECT OF NOCODAZOLE ON STIMULATION OF SHARK RECTAL GLAND BY VIP AND CNP.

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The rectal gland of *Squalus acanthias* can be stimulated to secrete chloride by two endogenous hormones, vasoactive intestinal peptide(VIP) and C-type natriuretic peptide (CNP). These agonists entrain different intracellular second-messenger cascades. VIP activates adenylate cyclase and protein kinase A, whereas CNP activates guanylate cyclase and probably protein kinase C. Direct stimulation of perfused glands by CNP is blocked by cytochalasin D, which interferes with actin filament organization, and by ML-7, which inhibits myosin light chain kinase, but these agents do not inhibit chloride secretion stimulated by VIP. It seems reasonable therefore to hypothesize that the actin cytoskeleton plays a larger role in transducing stimulation of the rectal gland induced by CNP than by VIP.

In the present experiments we attempted to explore the role of the microtubular portion of the cytoskeleton in chloride transport stimulated by VIP and CNP, by using the pharmacological agent nocodazole (Sigma Chemical Co). Nocodazole binds and disrupts intracellular microtubules in a reversible way, so that it is feasible to study its effect during applications and after washout.

Isolated rectal glands of *Squalus acanthias* were perfused with oxygenated shark Ringer's solution at pH 7.6 as previously described (Silva, P. et al. Methods Enzymol 192:754-66, 1990). Procaine, 20 mM, was added to all perfusion media in order to block the release of VIP from nerves within the gland, an action of natriuretic peptides that might otherwise complicate evaluation of the direct effect of CNP on rectal gland epithelial cells. In experiments with nocodazole the concentration of this compound in the perfusate was 20 μM. Duct fluid was collected at 10 minute intervals in small tared plastic centrifuge tubes and the volume measured every 10 minutes by weighing. The concentration of chloride in the duct fluid was estimated by amperometric titration.

An initial thirty minutes of control perfusion (three collection periods) allowed the gland to reach a stable basal state. At the end of this control period a 1 ml bolus of either VIP (Sigma Chemical Co.) or C-type natriuretic peptide (Sigma Chemical Co.) was perfused directly into the arterial catheter over 1 min. After three ten minute periods, the perfusion solution was changed to one without nocodazole. After an additional 30 minutes of washout, another identical bolus of VIP or CNP was injected into the rectal gland artery and duct secretion was collected for 3 final ten minute periods. It was thus possible to estimate the effect of VIP and CNP on the rectal gland secretion with and without nocodazole, and after 30-60 minutes of nocodazole washout.

The results are summarized in Figures 1 and 2. Perfusion with 20 μM nocodazole completely inhibited direct stimulation of the perfused rectal glands by CNP, and the inhibition was not relieved by nocodazole washout. Nocodazole partially inhibited, but did not prevent, stimulation by VIP, and this inhibition was reduced by nocodazole washout. Nocodazole consistently reduced the flow of perfusate to isolated glands, by and average of 32±4%, and the flow returned to normal levels during washout. This action is consistent with the known vasoconstrictive effect of microtubule depolymerization in mammalian vascular smooth muscle cells (Johns, D.G. et al. J. Biomed. Sci., 2000, 7: 1-43).

These results strengthen the hypothesis the the cytoskeleton is involved in the immediate stimulation of chloride secretion by the shark rectal gland. Disruption of the microtubular network inhibits secretion stimulated by CNP and VIP but the inhibition is more pronounced with CNP-induced stimulation. Supported in part by MDIBL and NIEHS P30 ESO3828-16.

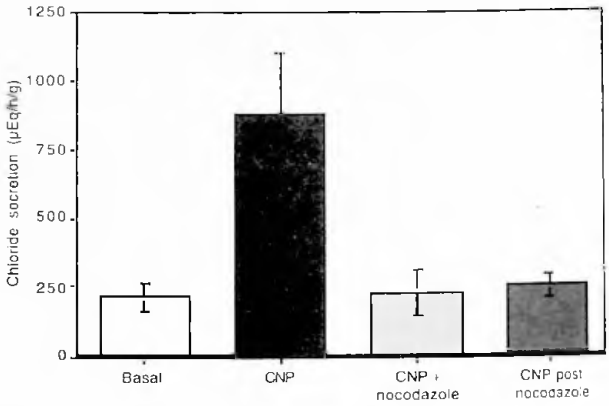

Figure 1. Effect of nocodazole on stimulation of perfused glands by CNP. Columns represent the average of 3 ten minute collection periods ± standard error of the mean. CNP was given over one minute as a bolus at a final concentration of 5 x 10^{-7}M. n= 5 for all columns. Nocodazole completely inhibited stimulation by CNP when present in the perfusion at a concentration of 20 μM, and after 30 minutes of washout. Values are mean±SEM.

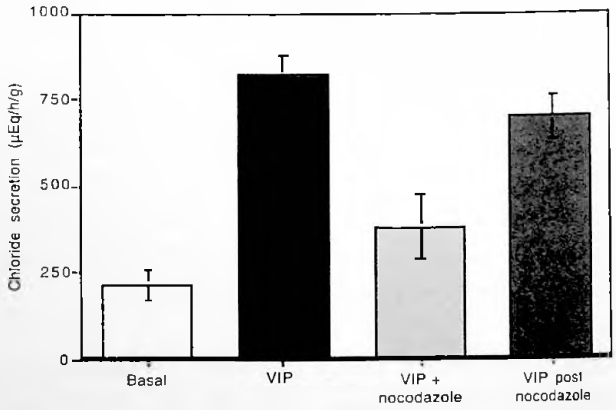

Figure 2. Effect of nocodazole on stimulation of perfused glands by VIP. Columns represent the mean ± standard error of the mean of three consecutive 10 minute periods before (Basal) and after the infusion over 1 minute of 10^{-7}M VIP (final concentration). Nocodazole partially inhibited stimulation by VIP, ($p<0.01$) and the inhibition was relieved during washout ($p< 0.02$). n=6 for basal and VIP and n=8 for VIP plus nocodazole and VIP post-nocodazole. Values are mean±SEM.