Further evidence that protein kinase C mediates stimulation of the secretion of chloride by CNP in the rectal gland of *Squalus acanthias*

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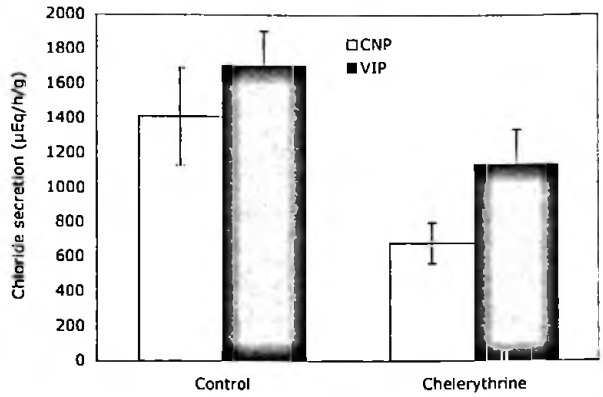
The stimulation of chloride secretion by C-type natriuretic peptide in the rectal gland of *Squalus acanthias* is mediated by both direct and indirect pathways. The indirect pathway involves the release of vasoactive intestinal peptide (VIP) from nerves within the gland, activation of adenylyl cyclase by VIP with the subsequent generation of cyclic-AMP and activation of protein kinase A. Previous work from our laboratory has suggested that the direct pathway of stimulation by CNP involves the activation of protein kinase C, because staurosporine inhibited CNP-induced stimulation in the presence of procaine. In the present series of experiments we used highly specific inhibitors of protein kinase C, chelerythrine and bisindolylmaleimide, to evaluate this question.

Isolated rectal glands of *Squalus acanthias* were perfused with oxygenated shark Ringer's solution at pH 7.6 as previously described.[1] 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 using a Buchler-Cotlove chloridrometer. Chelerythrine, 2μM, or bisindolylmaleimide, 0.05 and 0.1 μM were added to the perfusate at the beginning of the experiment whenever they were used. 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 CNP, calculated to deliver a concentration of 5×10^{-7}M to the gland, was injected directly into the arterial catheter over 1 min. In all experiments with CNP the perfusate contained procaine 10^{-2} M to prevent the release of VIP from the nerves within the gland. In experiments where VIP was used it was infused over one minute in an amount calculated to give a final concentration of 10^{-7} M. No procaine was used in the VIP experiments. The results are expressed as mean ± SEM.

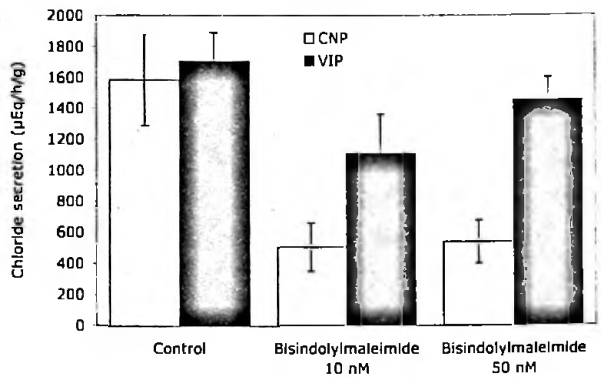
The results are summarized in Figure 1 and 2. Perfusion with chelerythrine 2×10^{-6}M inhibited the stimulatory effect of CNP on the secretion of chloride by the rectal gland by 50% from a peak increment of 1411 ± 282 μEq/h/g, n=7, to 680 ± 118, n=6, $p < 0.01$. Chelerythrine had no significant effect on the stimulation of chloride secretion by VIP; the peak increase in chloride secretion with VIP was 1705 ± 220 without chelerythrine, n=10, and 1136 ± 226 in its presence, n=6, $p > 0.05$.

Figure 1. Effect of chelerythrine on the secretion of chloride stimulated by CNP and VIP. Chelerythrine inhibits the stimulation by CNP but not that by VIP. Columns are mean ± SEM.



Bisindolylmaleimide inhibited the peak stimulation with CNP from 1583 ± 293, n=7, to 508 ± 155, n=6, with 0.1 μM bisindolylmaleimide ($p < 0.01$), and 541 ± 137, n=6, with 0.05 μM ($p < 0.01$). Bisindolylmaleimide had no effect on the stimulation induced by VIP. Peak response in control experiments was 1705 ± 186, n=10, 1112 ± 250, with 0.1 μM bisindolylmaleimide, n=4, $p > 0.05$, and 1455 ± 144 with 0.05 μM, n=7, $p > 0.05$.

Figure 2. Effect of bisindolylmaleimide on the secretion of chloride stimulated by CNP and VIP. Bisindolylmalei- mide inhibits the stimulation by CNP but not that by VIP. Columns are mean ± SEM.



These experiments support the hypothesis that the direct effect of CNP to stimulate the secretion of chloride by the rectal gland is mediated at least in part by protein kinase C. Previously this postulate had relied on the inhibition of the effect of CNP by staurosporine. That two different selective inhibitors of protein kinase C, chelerythrine and bisindolylmaleimide inhibit significantly the stimulatory effect of CNP but not that of VIP, strongly suggests that stimulation of the secretion of chloride by the rectal gland is mediated by two different pathways, one activated by VIP and mediated by cAMP/protein kinase A, and another activated by CNP and mediated by protein kinase C.

1. **Silva P., R. J. Solomon, F. H. Epstein**. Shark rectal gland. *Methods Enzymol.* 192;754-66, 1990.