Protein kinase C modulates the activation of the secretion of chloride mediated by protein kinase A in the rectal gland of the shark, *Squalus acanthias*.

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The secretion of chloride by the shark rectal gland is normally activated by the release of CNP from the heart in response to volume stimuli.[5] Circulating CNP causes the release of vasoactive intestinal peptide (VIP) from vipaminergic nerves within the rectal gland and, jointly with it, activates the secretion of chloride by the gland. These two peptides use different intracellular pathways to activate the final common pathway, the shark version of cystic fibrosis transmembrane regulator (CFTR).[4] VIP activates adenylate cyclase with the production of cAMP and subsequent activation of protein kinase A (PKA). The effect of CNP is mediated by protein kinase C but also requires the activation of guanylate cyclase and the production of cGMP.[4] The regulatory domain of CFTR has binding sites for both PKA and PKC and it can be activated *in vitro* by either of these two kinases.[2] Given that both these pathways share a common final pathway, CNP and VIP should be expected to have an additive effect on chloride secretion by the gland. Indeed, Aller et al. showed that the secretion of chloride induced by CNP can be potentiated by a small dose of VIP or forskolin.[1] In the present experiments we examined the nature of the relation between the VIP- and CNP-activated intracellular pathways.

Shark rectal glands were perfused as previously described.[3] Duct fluid was collected at 10-minute intervals in tared plastic centrifuge tubes and the volume assessed by weight. The concentration of chloride in the samples was measured by amperometric titration using a Buchler-Cotlove chlorhidrometer. All glands were perfused with shark-Ringers containing in addition, glucose 5 mM, and in the experiments with CNP, procaine 10^{-2}M to prevent release of endogenous VIP from the rectal gland nerves. Initial ten-minute control periods for the first 30-40 minutes of perfusion were collected to allow the gland secretion to reach a steady state. At the end of the control period a continuous infusion containing CNP, forskolin, dibutyryl cAMP, sildenafil, phorbol ester, or nitroprusside, alone or in combination, were started, and the experiment continued for an additional sixty minutes. When VIP was used it was either infused as a constant solution at a concentration of 10^{-9}M or given as a bolus in 1 ml of shark Ringer's solution infused directly into the rectal gland artery over one minute containing an amount calculated to deliver a final concentration of 10^{-7}M or 10^{-9}M to the gland.

Perfusion with CNP 5 x 10^{-9}M enhanced the response to a bolus infusion of VIP 10^{-9}M but had no effect on that of VIP at 10^{-7}M. Figure 1 shows the increment in the secretion of chloride above the control level after the bolus infusion of VIP. A constant infusion of VIP 10^{-9}M did not enhance the effect of an additional bolus of VIP 10^{-9}M. In fact, there was no additional response to the bolus (baseline 179±55; increment after the infusion of VIP 599±148, $p < 0.01$; increment after the additional VIP bolus 108±86, not significant, n=6).

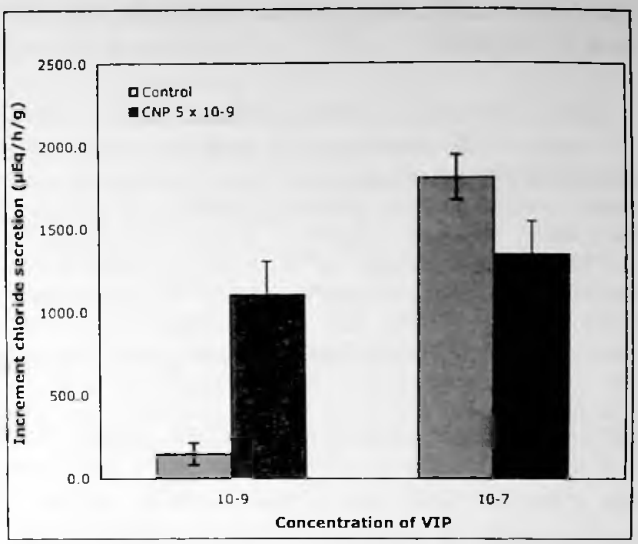

Figure 1. CNP potentiates the effect of VIP. In the glands perfused with CNP, an infusion of CNP 5 x 10^{-9}M was started thirty minutes after the beginning of the perfusion. Thirty minutes later a bolus of VIP, calculated to deliver a final concentration of 10^{-9}M, was injected directly into the artery over the course of one minute. In the control experiments, without CNP, the bolus of VIP was given thirty minutes after the start of the perfusion. CNP increased the secretory response of the gland seven-fold, control n=12, CNP n=7, p< 0.001. CNP had no enhancing effect when the concentration of VIP was 10^{-7}M, control n=20, CNP n=7, NS.

An infusion of CNP 5 x 10^{-9}M also enhanced the effect of dibutyryl cyclic AMP (dbcAMP), an analog of the intracellular messenger for VIP. Figure 2 shows the effect on chloride secretion of perfusion with CNP 5 x 10^{-9}M, dibutyryl cAMP 5 x 10^{-4}M, and the combination of both. The combination of CNP and cAMP had a significantly greater effect than CNP alone and a much faster effect than that of dbcAMP alone.

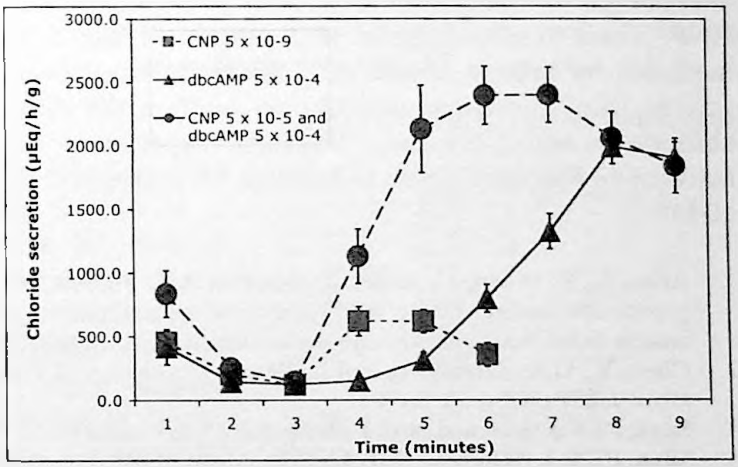

Figure 2. CNP potentiates the effect of dbCAMP. After a control period of thirty minutes, glands were perfused with CNP 5 x 10^{-9}M, with dbCAMP 5 x 10^{-4}M, or the combination of both. CNP increased the secretion of chloride approximately five times, n=12, $p < 0.001$. Dibutyryl cAMP increased the secretion of chloride sixteen times but the effect was delayed by about twenty minutes, n=6, $p < 0.001$. The combination of CNP and dbcAMP had an immediate effect that reached a similar peak thirty minutes earlier than that of dbcAMP alone, n=5, $p < 0.001$.

CNP activates protein kinase G and C, therefore, we examined the effect of cGMP and phorbol ester, activators of protein kinases G and C respectively on the stimulation induced by VIP. This combination had no effect on the secretory response to VIP (baseline 100±29; increment after the infusion of the combination of cGMP and phorbol ester, 399±91, $p < 0.01$; increment after the addition of a bolus of VIP 214±118, not significant, n=5). To examine further the effect of activation of protein kinase C and G on the effect of protein kinase A, the glands were perfused with a combination of nitroprusside 2.5 x 10^{-6}M, sildenafil 10^{-6}M, to activate guanylate cyclase and inhibit phosphosdiesterase V, with the purpose of activating protein kinase G; phorbol ester 10^{-6}M, to activate protein kinase C; and forskolin 10^{-7}M to activate protein kinase A. Figure 3 shows that at a concentration of 10^{-7}M forskolin induced a modest but significant stimulation of the secretion of chloride, n=7, $p < 0.05$. The combination of nitroprusside, sildenafil and phorbol ester enhanced the secretion of chloride about as much as CNP alone, n=4, $p < 0.025$. The addition of forskolin to the

combination of nitroprusside, sildenafil and phorbol ester markedly increased the secretion of chloride, n=4, $p < 0.005$.

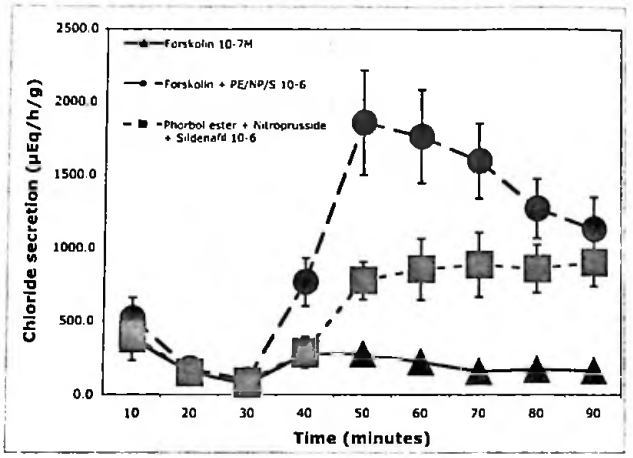

Figure 3. The combination of phorbol ester, nitroprusside and sildenafil potentiates the effect of forskolin. After a control period of thirty minutes, glands were perfused with forskolin 10^{-7}M, with phorbol ester 10^{-6}M, nitroprusside 2.5×10^{-6} M, and sildenafil 10^{-6}M, or the combination of both. Forskolin increased the secretion of chloride approximately 4 times, n=7, $p < 0.05$. The combination of phorbol ester, nitroprusside, and sildenafil increased the secretion of chloride ten-fold, n=4, $p < 0.001$. The combination of phorbol ester plus nitroprusside and sildenafil and forskolin stimulated the secretion of chloride nineteen times, n=4, $p < 0.001$.

In all of these experiments, activation of protein kinase A simultaneously with or after activation of protein kinase C resulted in increases in the secretion of chloride beyond those expected of an additive effect. CNP enhanced to response to VIP seven-fold. The combination of nitroprusside, sildenafil and phorbol ester enhanced the effect of forskolin five times. The effect of CNP on that of dbCAMP was more complicated; during the first thirty minutes of perfusion CNP increased the effect of dbcAMP almost eight-fold, but after an hour of perfusion the effect of dbCAMP alone was the same as that with CNP. These results suggest that protein kinase C modulates the activation of chloride transport mediated by protein kinase A. This modulatory enhancement of the effect of protein kinase A by activation of protein kinase C may explain the dual mechanism of activation of the secretion of chloride by both CNP and VIP. These experiments also confirm previous experiments showing that activation of protein kinase C requires the concurrent activation of guanylate cyclase and production of cGMP.

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