Sildenafil citrate enhances the stimulation of the secretion of chloride by CNP in *Squalus acanthias* rectal gland

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C-type natriuretic peptide (CNP) stimulates the secretion of chloride by the rectal gland of *Squalus acanthias* by both direct and indirect pathways. The indirect pathway involves the release of vasoactive intestinal peptide (VIP) from nerves within the gland and activation of adenylyl cyclase by VIP with the subsequent generation of cyclic-AMP and activation of protein kinase A. The direct effect involves, at least in part, activation of guanylyl cyclase and the generation of cyclic-GMP. Our previous experiments have shown that infusion of 8-bromo-cyclic GMP alone or in combination with zaprinast (an inhibitor of phosphodiesterase 5) fails to evoke stimulation of the secretion of chloride in isolated perfused rectal glands.[1] Among the possible explanations for the failure to evoke stimulation with this combination are that 8-Br-cGMP does not easily permeate the cellular membranes of the rectal gland cells, or that zaprinast is not an effective inhibitor of phosphodiesterase V in the rectal gland. In the present experiments we used sildenafil citrate, a specific inhibitor of phosphodiesterase 5, to determine whether inhibition of this enzyme potentiates the direct effect of CNP to stimulate the secretion of chloride in the rectal gland.

Isolated rectal glands of *Squalus acanthias* were perfused with oxygenated shark Ringer's solution at pH 7.6 as previously described.[2] 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. 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 the perfusate was changed to a solution containing sildenafil citrate 10^{-4}M. Ten minutes later a 1 ml bolus of CNP, calculated to deliver a concentration of 1.2 or 5 x 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 Figures 1 and 2. Perfusion with sildenafil citrate 10^{-4}M potentiated and prolonged the stimulatory effect of CNP on the secretion of chloride by the rectal gland. Importantly, exposure to sildenafil alone did not stimulate the gland or prevent the usual decline in chloride secretion during the initial thirty minutes of perfusion. When CNP was injected at a concentration of 1.2 x 10^{-7}M in the presence of sildenafil citrate, the secretion of chloride was stimulated to a rate four times greater than when CNP was infused alone (Figure 1). In the experiments where CNP was infused at a concentration of 5 x 10^{-7}M, sildenafil citrate substantially prolonged the effect of CNP, (Figure 2). The effect of sildenafil citrate appears to be specific for phosphodiesterase 5

and CNP because it did not enhance stimulation by VIP which is mediated by cAMP. Sildenafil citrate did not enhance or prolong the effect of VIP (Figure 3).

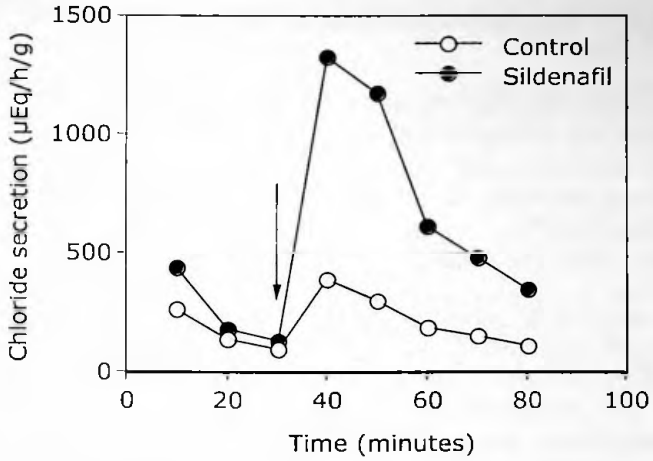


Figure 1. Effect of sildenafil citrate 10^{-4}M on stimulation of perfused glands by CNP 1.2 x 10^{-7}M. CNP was injected into the rectal gland artery at the time indicated by the arrows. Sildenafil enhanced the stimulation of chloride secretion by CNP. Values are mean ± SEM, n=6 for both groups.

Figure 2. Effect of sildenafil citrate 10^{-4}M on stimulation of perfused glands by CNP 5 x 10^{-7}M. CNP was injected into the rectal gland artery at the time indicated by the arrows. Sildenafil prolonged the stimulation of chloride secretion by CNP. Values are mean ± SEM, n=7 and 4 for control and sildenafil groups, respectively.

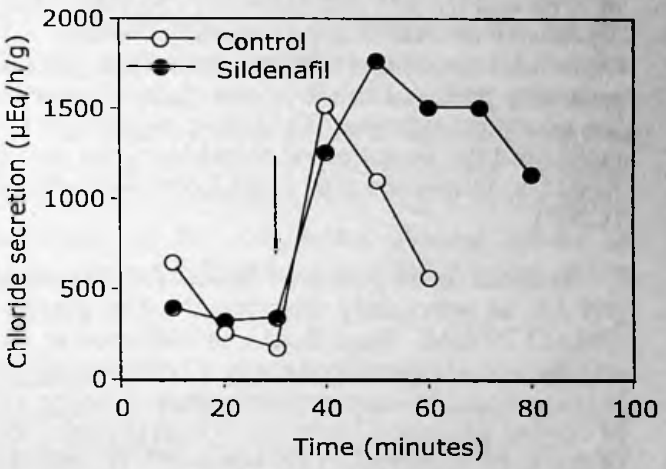
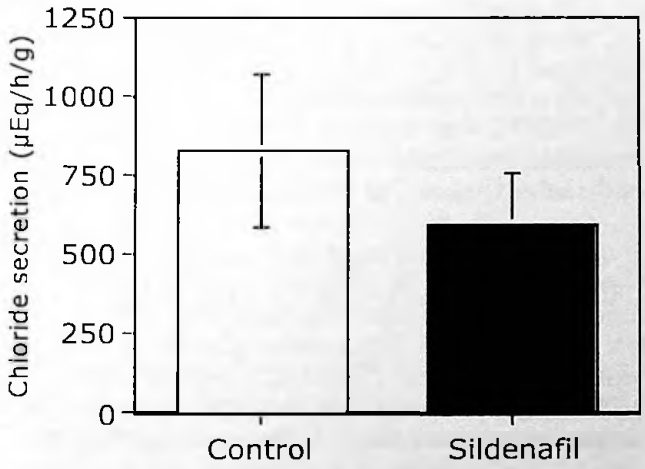


Figure 3. Effect of sildenafil citrate 10^{-4}M on stimulation of perfused glands by VIP 10^{-7}M. The columns show the increment in the secretion of chloride after the administration of VIP in the presence and absence of sildenafil. Sildenafil did not enhance the stimulation of chloride secretion by VIP. Values are mean±SEM, n=8 and 6 for control and sildenafil groups, respectively.



These experiments provide the first functional evidence that cGMP mediates the direct effect of CNP to stimulate the secretion of chloride by the rectal gland. Inhibition of phosphodiesterase 5, which hydrolyzes cGMP, potentiates and prolongs the effect of CNP. We have previously shown that natriuretic peptides activate guanylyl cyclase in the rectal gland of the shark. These results provide indirect evidence that the effect of CNP to stimulate the secretion of chloride is mediated at least in part by cGMP, generated by activation of guanylyl cyclase.

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2. Silva P, Solomon RJ, and Epstein FH. Shark rectal gland. In: *Methods Enzymol.* 192:754-766, 1990