MECHANISM OF BOMBESIN INHIBITION OF STIMULATED CHLORIDE SECRETION BY THE RECTAL GLAND OF Squalus acanthias.

Patricio Silva, Patricio Silva, Jr, Judd Landsberg, Marcela Silva
S. Reichlin and Franklin H. Epstein
Department of Medicine Beth Israel Hospital and Harvard Medical School, Boston, MA 02215

We have previously shown that bombesin, a tetradecapeptide present in nerve fibers in the rectal gland of the shark together with VIP (Cell Tissue Res 234:595, 1983), inhibits VIP and cyclic AMP stimulated chloride secretion by the perfused rectal gland of Squalus acanthias (Bull. MDIBL 27:51-53, 1987-1988). This effect of bombesin to inhibit chloride secretion at a site distal to the generation of cyclic AMP is reminiscent of that previously demonstrated for somatostatin, also present in the nerves within the gland. In the present series of experiments we examined the mechanism of action of bombesin.

Isolated rectal glands were perfused as previously described (Am J Physiol 233:F298, 1977). The glands were perfused for 90 minutes with a continuous infusion of VIP 1.5 x 10^{-9} M to obtain sustained chloride secretion. Thirty minutes after starting the perfusion bombesin was added to the perfusate at a final concentration 8 x 10^{-7} M. The perfusion with bombesin was continued for thirty minutes followed by additional thirty minutes of perfusion with VIP alone. Bombesin reversibly reduced the secretion of chloride induced by VIP by 56.5.±9.7% (n=7, $p<0.01$). A representative experiment is shown in Figure 1.

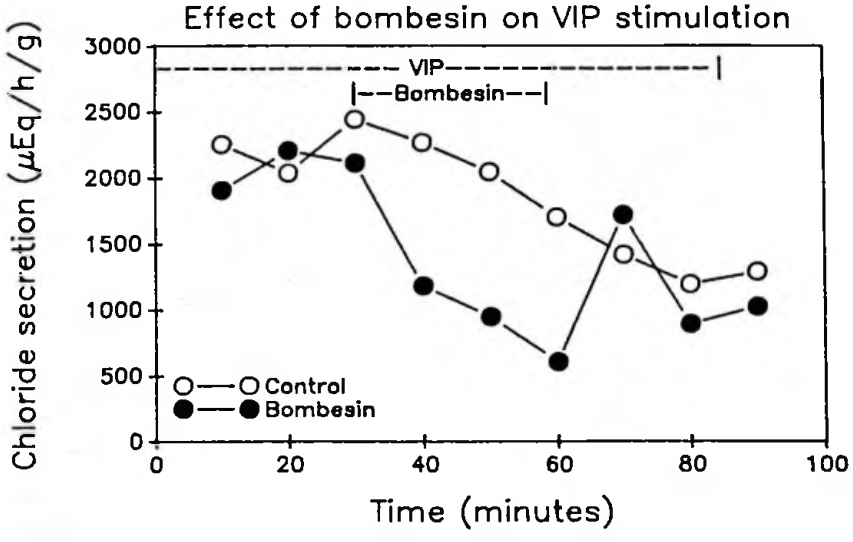


Figure 1. This graph is a representative experiment of the effect of bombesin to inhibit stimulation by VIP. VIP was infused continuously at a concentration of 1.5 x 10^{-9} M. Bombesin was administered for 30 minutes during the period marked by the vertical lines at a final concentration of 8 x 10^{-7} M.

Because of the similarity of the effect of bombesin to that of somatostatin we measured the level of somatostatin in the venous effluent of the rectal gland during the perfusion with bombesin. Perfusion with bombesin evoked an increase in the concentration of somatostatin in the venous effluent of the gland, from 30.6±5.7 pg/ml in controls to 215±20.8 after bombesin, n=4, $p<0.01$.

To explore further the mechanism of inhibition of chloride secretion by bombesin glands were perfused with procaine 10^{-2} M to inhibit the release of neurotransmitters from the nerve endings within the gland. Procaine completely prevented the effect of bombesin while it did not have any effect of its own. Representative experiments are shown in Figure 2. This results

were corroborated using nifedipine to inhibit neurotransmitter release. Representative results are shown in figure 3. Nifedipine also suppressed the inhibitory effect of bombesin.

Figure 2. Effect of procaine on bombesin inhibition. Procaine 10^{-2}M prevented inhibition by bombesin while it did not have an effect of its own. Three representative experiments

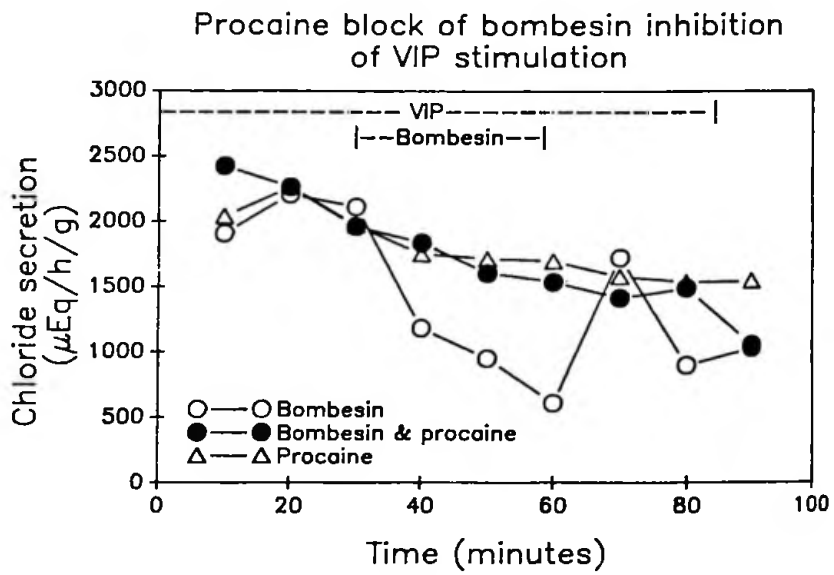
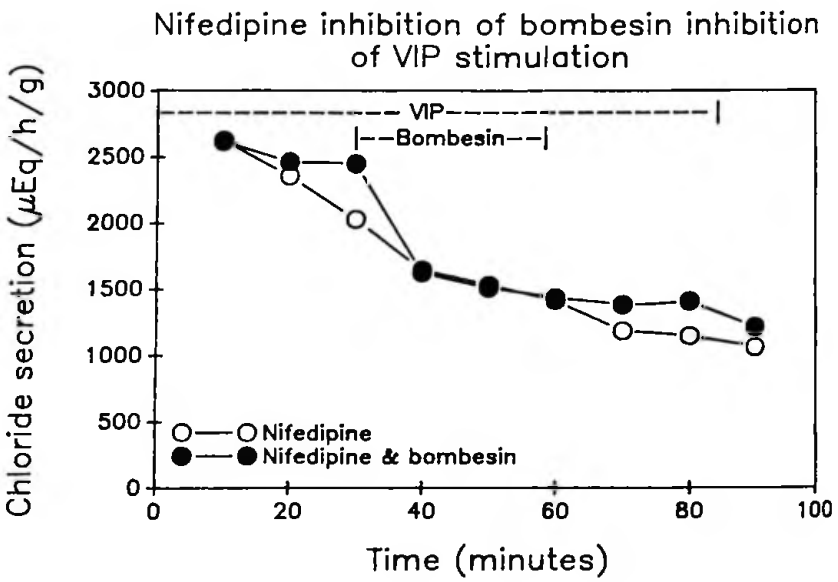
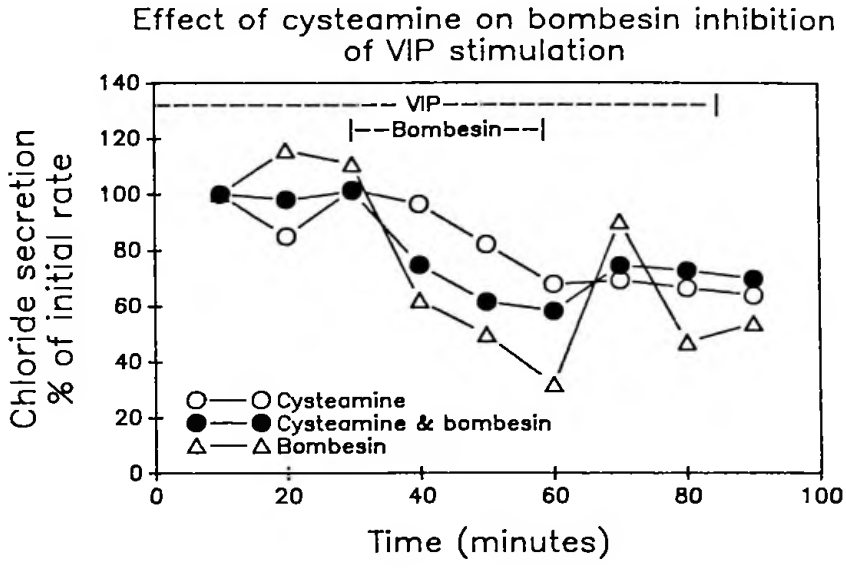


Figure 3. Nifedipine 10^{-6}M completely prevented the effect of bombesin to inhibit VIP stimulation. Two representative experiments.



The effect of bombesin to release somatostatin was explored in yet another way. Cysteamine breaks the sulfhydryl bond in the somatostatin molecule rendering it ineffective. Perfusion with cysteamine would be expected to reduce the inhibitory effect of bombesin if the latter is due to the release of somatostatin. The results of the perfusions with cysteamine are shown in figure 4. Cysteamine partially suppressed the effect of bombesin in agreement with its effect to inactivate somatostatin.

Figure 4. Representative experiments showing the effect of cysteamine on the inhibition by bombesin of VIP stimulation. Cysteamine was infused at a concentration of 200 mg/l. Cysteamine inactivates somatostatin.



Bombesin is present together with VIP, somatostatin and cholecystokinin in the nerve fibers within the rectal gland. Bombesin inhibits stimulated chloride secretion at a site distal to that of the generation of cyclic AMP by the liberation of somatostatin from nerve endings within the gland. It is attractive to postulate that nerve stimulation or increase in circulating ANP would start a cascade of effects that cause locally regulated increase in chloride secretion. Bombesin may be released together with VIP from the nerve endings in response to nerve stimulation or to increases in circulating ANP. Bombesin would cause the subsequent release of somatostatin from the nerve fibers. Somatostatin would then inhibit the secretion of chloride.

The observations reported here support the notion that the activity of the rectal gland is normally regulated by the release of endogenous neurotransmitters.

This work was supported by grants provided by the USPHS NIHDK18078 and NIHHL35998, and NSF DCB850826.