FAILURE OF VIP ANTAGONISTS TO INHIBIT THE EFFECT OF VIP ON CHLORIDE SECRETION BY THE RECTAL GLAND OF SQUALUS ACANTHIAS

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The stimulatory effect of CNP is partially inhibited by procaine 10^{-2} M, which suppresses the release of VIP, suggesting that CNP, like ANP, stimulates chloride secretion, at least partially, by the release of VIP (Solomon, R., et al. Bull. MDIBL 1993). CNP stimulates short circuit current (Isc) in confluent monolayers of primary cultures of rectal gland cells mounted in Ussing chambers and increases oxygen consumption by separated rectal gland tubules indicating that CNP, unlike ANP, has a direct stimulatory effect on chloride secretion by the rectal gland. Thus, CNP appears to have a dual mechanism of action comprising direct and indirect effects to stimulate the secretion of chloride by the rectal gland. To unravel the contribution of VIP to the stimulatory effect of ANP and CNP it would be desirable to be able to block the stimulatory effect of VIP on chloride secretion. To this end we tested the effect of a number of putative VIP antagonists in isolated perfused rectal glands. The glands were perfused using a technique described previously (Silva, P., et al., Methods Enzymol. Vol 192, 1990:754-66). The following five peptides were used: VIP 1-12 (1-12); VIP 10-28 (10-28); [^{4}Cl-d-phenylalanine^{6}, leucine^{17}]-VIP (4CldP6L17) (Pandol, S.J., et al., Am. J. Physiol. 250:G553, 1986); [lysine^{1},proline^{2,5}, arginine^{3,4}, tyrosine^{6}]-VIP (KPRY) (Gozes, I. et al., Endocrinol. 125:2945, 1989); and [acetyl-tyrosine^{1}, d-phenylalanine^{2}]; and [acetyl-tyrosine^{1}, d-phenylalanine^{2}] GHRF 1-29 amide (AcYdFGHRF) (Waelbroeck, M., et al., Endocrinol. 116:2643, 1985). The rectal glands were perfused for thirty minutes without peptides. During the following thirty minutes the glands were perfused with the peptides at a concentration of 1.5 x 10^{-8} M, ten-fold greater than the usual concentration of VIP. The results are shown in Figure 1. None of the peptides stimulated or inhibited chloride secretion. At the end of this thirty minute period, the perfusate solution was changed to one that contained in addition VIP 1.5 x 10^{-9} M. None of the peptides prevented the effect of VIP. Some had a minor inhibitory effect. We conclude that none of the available VIP antagonists either stimulate chloride secretion or can prevent in a ten-fold greater concentration the stimulatory effect of VIP. Therefore, they are not useful as probes of the effect of VIP in the rectal gland.

Figure 1. Effect of vasoactive intestinal peptide antagonists on VIP stimulation of chloride secretion. A sixty minute perfusion with the antagonists 1.5 x 10^{-8} M was started after 30 minutes of a control perfusion. After thirty minutes of perfusion with the antagonists alone VIP was added to the perfusate at a concentration of 1.5 x 10^{-9} M. None of the inhibitors either stimulated chloride secretion or prevented the effect of VIP. The number of experiments were 11, 5, 5, 4, 3, 4, in the order, top to bottom, of the legend in the graph. Error bars are omitted to avoid crowding of the figure.

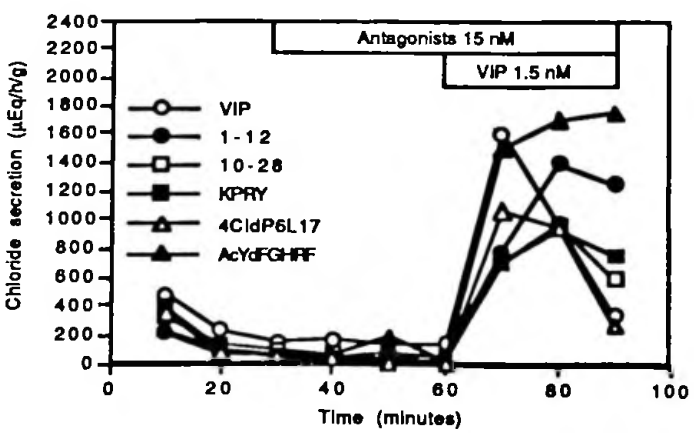

Figure 1.

These experiments provide additional information. The observation that the VIP fragments 1-12 and 10-28 had no stimulatory effect implies that the full length of the VIP molecule is necessary to stimulate secretion. The importance of the initial six amino acids in the amino terminal end is underlined by the failure of K^{1}-$P^{2,5}$-$R^{3,4}$-Y^{6} to stimulate chloride secretion. This peptide has all six initial amino acids replaced. Chlorination of the phenylalanine in position 6 and replacement of the methionine in position 17 with a leucine as in ClF^{6}-L^{17} eliminates all stimulatory effect indicating that chlorination or the minor replacement of a methionine by a leucine renders the peptide inactive.

VIP	H-S-D-A-V-F-T-D-N-Y-T-R-L-R-K-Q-M-A-V-K-K-Y-L-N-S-I-L-N
VIP 1-12	H-S-D-A-V-F-T-D-N-Y-T-R
VIP 10-28	Y-T-R-L-R-K-Q-M-A-V-K-K-Y-L-N-S-I-L-N
ClF^{6}-L^{17}-VIP	H-S-D-A-V-**F**-T-D-N-Y-T-R-L-R-K-Q-**L**-A-V-K-K-Y-L-N-S-I-L-N **Cl**
K^{1}-$P^{2,5}$-$R^{3,4}$-Y^{6}-VIP	**K-P-R-R-P-Y**-T-D-N-Y-T-R-L-R-K-Q-M-A-V-K-K-Y-L-N-S-I-L-N
AcY^{1}-GHRF	**Y-F**-D-A-**I**-F-T-**N-S**-Y-**R-K-V**-L-**G**-Q-**L-S-A-R**-K-**L**-L-**Q**-D-I-**M-S-R** **Ac**

Figure 2. Aminoacid sequence of the VIP antagonists used in these experiments. The aminoacids that differ from the parent molecule are depicted in bold-type.

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