FUNCTIONAL CHARACTERIZATION OF THE VIP RECEPTOR IN THE RECTAL GLAND OF SQUALUS ACANTHIAS

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We have previously examined the functional characteristics of the vasoactive intestinal peptide (VIP) receptor of the rectal gland using putative inhibitory peptides that have been used as antagonists of VIP in several different tissues (Silva, P, et al. Bull MDIBL 32:78-79, 1993). In those experiments, all the antagonists used at a molar ratio inhibitor/VIP greater than ten failed to inhibit the effect of VIP. In a continued effort to characterize the VIP receptor of the rectal gland we chose to examine the stimulatory effects of several peptides that share similar sequences of amino acids with VIP. The peptides studied were helodermin, helospectin I, PACAP27 and PACAP38. The amino acid sequence of these peptides is shown in Table I. Helodermin and helospectin share 15 amino acids with VIP, and differ in 5 amino acids between them. Both are extended by 7 and 10 amino acids, respectively, in the amino terminal end. PACAP27 and PACAP38 differ in 9 amino acids with VIP, PACAP27 is one amino acid shorter than VIP while PACAP38 is 10 amino acids longer. They differ from helodermin and helospectin by 13 amino acids.

Table I
Amino acid sequences of peptides of the VIP family. Sequence homologies are underlined.

	1 10 20 30 38
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
Helodermin	H-S-D-A-I-F-T-Q-Q-Y-S-K-L-L-A-K-L-A-L-Q-K-Y-L-A-S-I-L-G-S-R-T-S-P-P-P
Helospectin I	H-S-D-A-T-F-T-A-E-Y-S-K-L-L-A-K-L-A-L-Q-K-Y-L-E-S-I-L-G-S-S-T-S-P-R-P-P-S-S
PACAP27	H-S-D-G-I-F-T-D-S-Y-S-R-Y-R-K-Q-M-A-V-K-K-Y-L-A-A-V-L
PACAP38	H-S-D-G-I-F-T-D-S-Y-S-R-Y-R-K-Q-M-A-V-K-K-Y-L-A-A-V-L-G-K-R-Y-K-Q-R-V-K-N-K

We examined the effect of these peptides on chloride secretion by the isolated perfused rectal gland, oxygen consumption by separated rectal gland tubules, and adenylyl cyclase activity in rectal gland plasma membranes. Rectal glands were perfused as previously described (Silva P, et al. Methods Enzymol. Vol. 192:754-66, 1990).

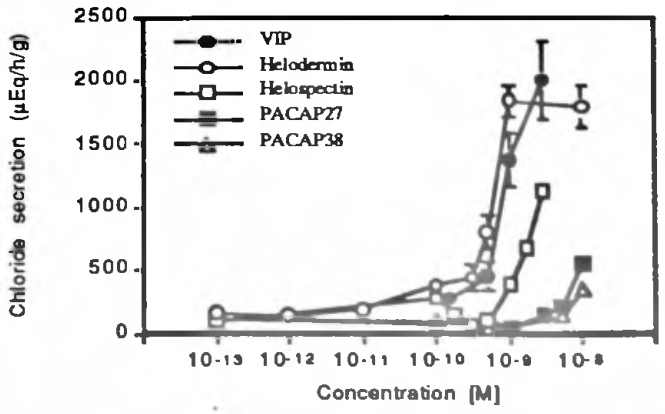

Figure 1. Chloride secretion by isolated perfused rectal glands in response to VIP-like peptides.

Figure 1 shows the effect of these peptides on chloride secretion by the rectal gland in comparison with that of VIP. The effect of helodermin was essentially similar to that of VIP while that of helospectin was one log unit lower than that of either VIP or helodermin. Both PACAPs were significantly lower, more than two log units, than VIP or helodermin and even helospectin.

To determine whether the effect of helodermin was exerted directly on the rectal gland cell and not mediated by the release of VIP from rectal gland nerves we examined its effect on oxygen consumption by separated rectal gland tubules. Tubules were prepared as previously described (Silva P, et al. Methods Enzymol. Vol. 192:754-66, 1990). Figure 2 shows the effect of helodermin on ouabain sensitive oxygen consumption. Helodermin stimulated oxygen consumption in a dose related way. Its dose response curve was displaced approximately 1 log units to the right when compared with the effect of VIP, and approximately 1.5 log units also to the right when compared with its effect on chloride secretion in perfused rectal glands.

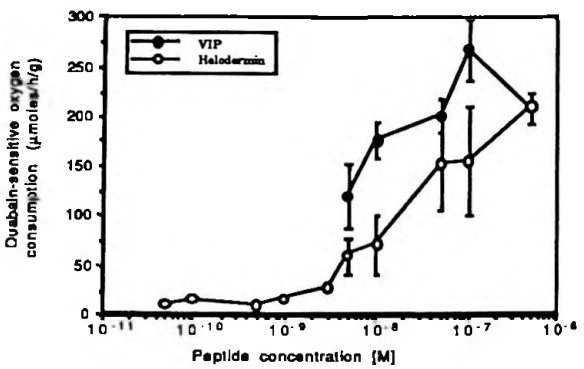

Figure 2. Effect of VIP and helodermin on oxygen consumption by separated rectal gland tubules.

To determine whether these peptides stimulate the secretion of chloride by activating adenylyl cyclase we measured the activity of adenylyl cyclase in plasma membranes of rectal gland cells. The preparation of plasma membranes and the measurement of the activity of adenylyl cyclase were done as previously described (Silva P, et al. Am. J. Physiol. 265:R439-46, 1993). The results are shown in figure 3. All peptides stimulated adenylyl cyclase in a dose related way. The order of potency was helodermin ≥ helospectin > VIP >> PACAP.

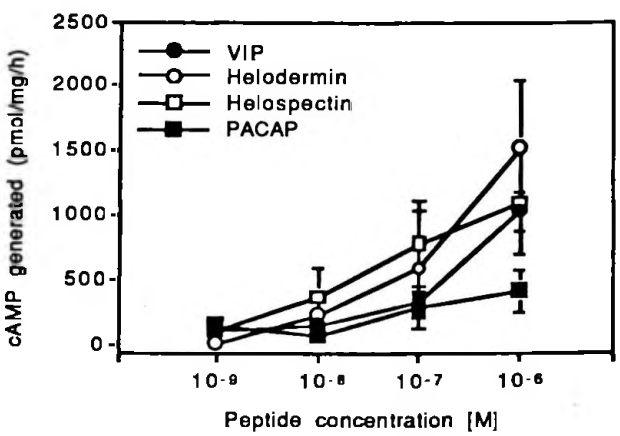

Figure 3. Effect of VIP-like peptides on rectal gland adenyl cyclase.

These results show that peptides that are similar in their amino acid sequence to VIP stimulate chloride secretion by the rectal gland of the shark and confirm the observation reported in the Bulletin by Stidham et al. for PACAP38 (Stidham JD, et al. Bull. MDIBL 33:85, 1994). The result are surprising because the peptides that are most different from VIP, helodermin and helospectin, have the same or very similar potency as VIP. PACAP peptides which are closer to VIP than helodermin and helospectin are substantially less active than VIP. These results offer an insight into the characteristics of the peptides that activate chloride secretion by the rectal gland. The loss of activity of PACAP as compared

with VIP indicates that changes in the initial and terminal sequences are important for the maintenance of activity. The initial and terminal sequences are conserved or have minor changes in helodermin and helospectin that have activities similar to that of VIP. Changes in the middle of the sequence do not appear to have any impact as judged by the fact that helodermin and helospectin, that differ mostly from VIP in the amino acids in positions between 8 and 20, retain activity. Elongating the chain beyond 28 amino acids to 35 amino acids, as in helodermin, does not affect potency, but further elongation to 31 amino acids, as in helospectin, reduces activity. These results, together with those previously reported (Silva, P, et al. Bull MDIBL 32:78-79, 1993) where fragments of the VIP molecule from the amino and the carboxy terminal were used without either stimulatory or inhibitory effects indicate that both ends of the molecule need to be present simultaneously for activation of the receptor to occur.

A stimulatory effect of these peptides was seen on oxygen consumption by separated rectal gland tubules, a preparation devoid of rectal gland nerves. Therefore, the effect of these peptides cannot be mediated by the release of another neurotransmitter from the cells as observed for the effect of atrial natriuretic peptide.

At a cellular level, the effect of these peptides is mediated by adenylyl cyclase as demonstrated by their capacity to activate this enzyme in plasma membranes preparations of rectal gland cells. These peptides exert their effect directly on the rectal gland cells by activating adenylyl cyclase.

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