Evidence that helodermin, a newly extracted peptide from Gila monster venom, is a member of the secretin/VIP/PHI family of peptides with an original pattern of biological properties

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Helodermin, a newly isolated peptide from the venom of Gila monster (*Heloderma suspectum*) was shown to stimulate the adenylate cyclase activity of rat pancreatic membranes as efficiently as secretin and VIP. It also increased cyclic AMP levels and inhibited [^{125}I]VIP binding in rat pancreatic acini. Finally, helodermin activated adenylate cyclase in membranes from rat heart, rat brain, and human heart, showing properties analogous yet distinct from those of secretin, VIP and PHI.

Gila monster venom *Helodermin* *Secretin* *Vasoactive intestinal peptide* *Bombesin* *Adenylate cyclase*

1. INTRODUCTION

A new peptide, called helodermin, has been purified to homogeneity from the venom of Helodermina lizards, based on its capacity to stimulate rat pancreatic adenylate cyclase [1]. Here we demonstrate that helodermin is able to: (i) stimulate, as efficiently as secretin and VIP, the adenylate cyclase activity of rat pancreatic plasma membranes; (ii) increase cyclic AMP levels and inhibit [^{125}I]VIP binding competitively on dispersed rat pancreatic acini; and (iii) stimulate adenylate cyclase activity in membranes from rat heart, rat brain, and human heart differently from secretin, VIP and PHI. We conclude that helodermin is a member of the secretin/VIP/PHI family of peptides that exhibits a pattern of biological activities analogous yet distinct from that of the 3 reference peptides.

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Abbreviations: VIP, vasoactive intestinal peptide; PHI, peptide having an N-terminal histidine and a C-terminal isoleucine amide

2. MATERIALS AND METHODS

Helodermin was purified as in [1]. The molar peptide concentration was estimated by two converging techniques. The protein content determined with the Folin reagent [2] assumed an M_r value of 5900.

Rat pancreatic acini were prepared and incubated as indicated in [3]. The acini were incubated at 37°C for 30 min. One ml cold ethanol was then added and the suspension was centrifuged for 30 min at 2000 × *g*. The supernatant was evaporated, and the residue was dissolved in 0.05 M acetate buffer (pH 6.2). Cyclic AMP was determined using a cyclic AMP ^{125}I RIA Kit (New England Nuclear, Dreieich). Under all conditions tested, cyclic AMP levels plateaued before 15 min.

Binding of [^{125}I]VIP to pancreatic acini was performed as follows. [^{125}I]VIP was prepared as in [4] and purified as in [5]. The radioactive ligand was

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added to the incubation medium enriched with 0.1 mg/ml bacitracin with or without an unlabelled peptide. After 1 h, the incubation suspension was layered on di-*n*-butylphthalate. The acini were sedimented through the dense layer by a 15-s centrifugation. The microfuge tube was frozen in liquid nitrogen and cut through the dense layer. The radioactivity in the lower part was counted in a gamma-counter. Non-specific binding referred to [^{125}I]VIP bound in the presence of 1 μM VIP. Total binding never exceeded 10% of the tracer offered and non-specific binding amounted to 20% of total binding.

Rat pancreatic plasma membranes were prepared by two different methods. The first one was identical to that in [6], the second one was similar, except for the omission of 5 mM 2-mercaptoethanol in all buffers used. It was verified that both methods gave identical profiles of membrane sedimentation.

Rat brain synaptic membranes, rat heart membranes, and human heart membranes were prepared as in [7–9].

Adenylate cyclase activity was determined as in [10] as described in [11]. Ten μM GTP was added systematically. Under all experimental conditions tested, the conversion of [α-^{32}P]ATP into cyclic [^{32}P]AMP was linear with time.

Synthetic porcine secretin and secretin-(7–27) were generous gifts from Dr W. König (Hoechst Aktiengesellschaft, Frankfurt/Main). Synthetic VIP was purchased from UCB Bioproducts (Brussels).

3. RESULTS

3.1. *Effects of helodermin on rat pancreatic preparations*

Helodermin inhibited dose-dependently and competitively the binding of [^{125}I]VIP to dispersed rat pancreatic acini. The peptide was 10 times less potent than unlabelled porcine VIP but 3 times more potent than porcine secretin (fig.1, left panel). In the same type of preparation, VIP and secretin exerted distinct effects on cyclic AMP levels (fig.1, right panel). The relatively flat curve of VIP stimulation contrasted with the steeper curve of secretin and the maximum effect of VIP was significantly lower than that of secretin. The efficacy of helodermin on this parameter was comparable to that of secretin but the potency of the peptide was 3 times lower than that of secretin.

Adenylate cyclase activity in rat pancreatic membranes prepared in the presence (fig.2, left panel) or absence (fig.2, right panel) of 5 mM 2-mercaptoethanol was differently stimulated by

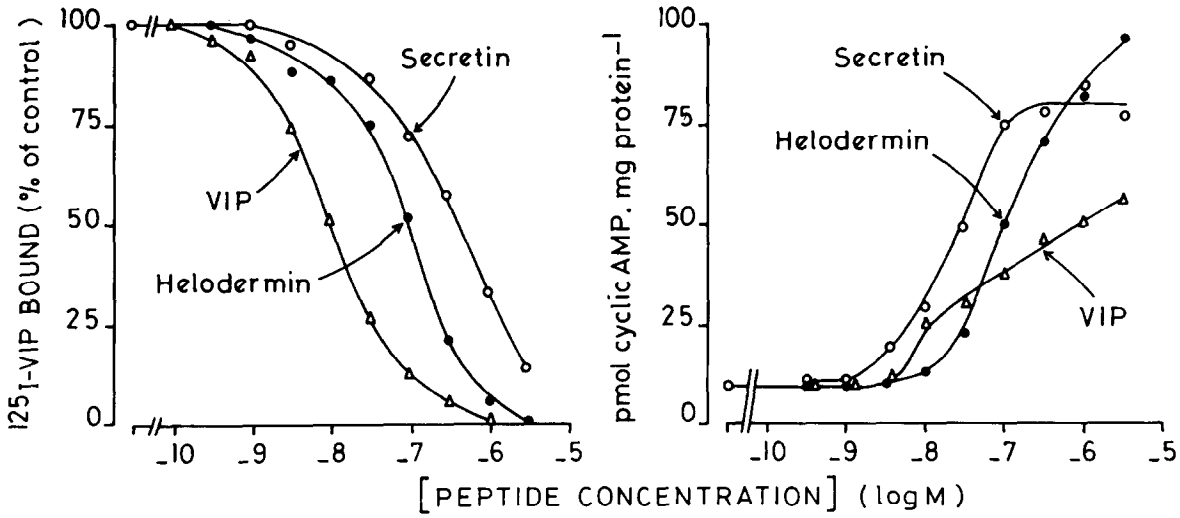

Fig.1. Dose–effect curves of VIP (△), helodermin (●) and secretin (○) inhibition of [^{125}I]VIP binding (left panel) and cyclic AMP formation (right panel) in rat pancreatic acini. The results were representative of two other experiments.

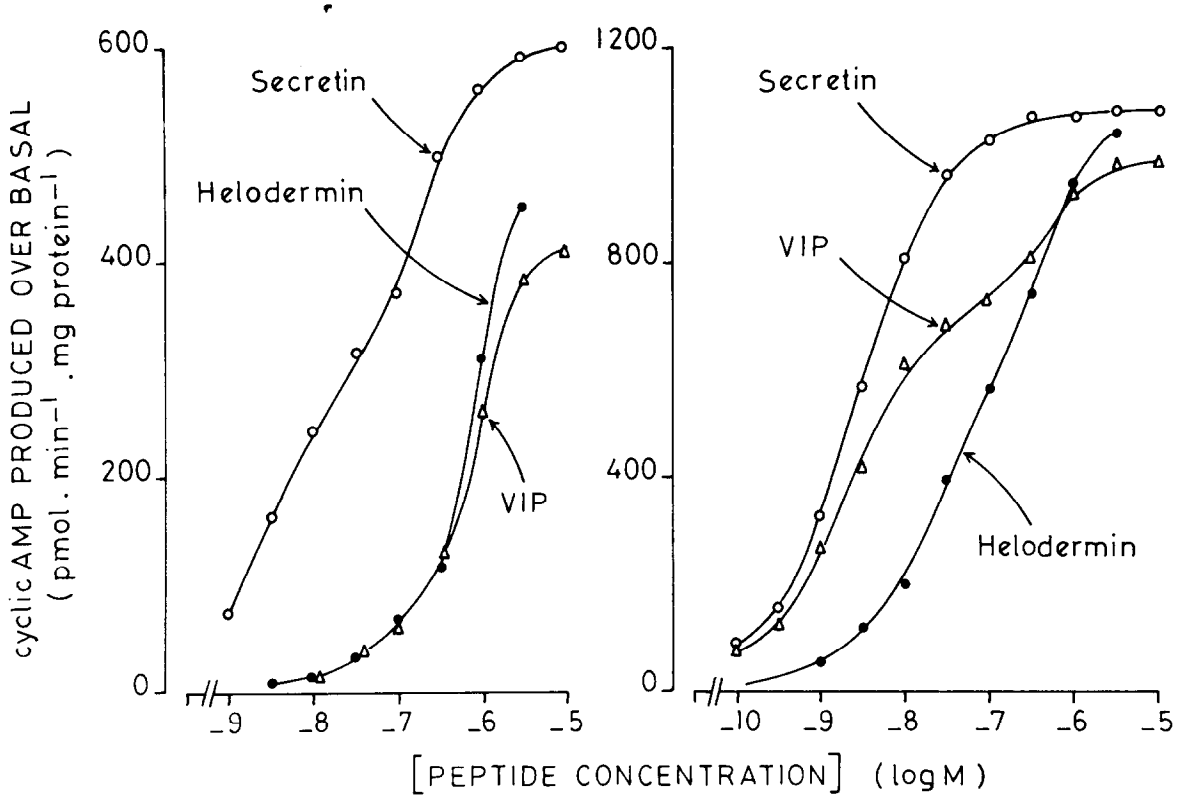

Fig.2. Dose–effect curves of adenylate cyclase activation by secretin (○), VIP (△) and helodermin (●) in rat pancreatic plasma membranes prepared in the presence (left panel) or absence (right panel) of 5 mM 2-mercaptoethanol. The data were expressed in pmol cyclic AMP produced · min^{-1} · mg $protein^{-1}$ and were representative of two other experiments. Basal activities were 23 and 43 pmol cyclic AMP · min^{-1} · mg $protein^{-1}$, respectively, in membranes prepared with and without 5 mM 2-mercaptoethanol.

secretin, VIP, and helodermin. As compared to membranes prepared without 2-mercaptoethanol, those prepared in the presence of the reducing agent showed a lower specific activity of the enzyme and, in addition, the sensitivity to secretin and helodermin was reduced 10-fold and that to VIP even further (200-fold). In this system, helodermin stimulation was, thus, more closely related to that of secretin than to that of VIP, although helodermin was 30-fold less potent than secretin. This hypothesis was further supported by the fact that secretin-(7–27), a secretin fragment previously reported to inhibit secretin effects competitively and selectively [11,12], was able to inhibit dose-dependently the helodermin-stimulated adenylate cyclase activity (fig.3). Similar results were obtained with the crude venom tested under the same conditions [13].

3.2. *Effects of helodermin on adenylate cyclase activity in membranes from human heart, rat heart and rat brain*

Adenylate cyclase activity in human heart membranes was stimulated by VIP but not by secretin. Helodermin was more potent (4-fold) and more efficient (by 25%) than VIP on this preparation (fig.4, left panel). In rat heart membranes, adenylate cyclase activity was stimulated by both secretin and VIP, although secretin was more potent and efficient than VIP. In this model, helodermin appeared to be less potent but nearly as efficient as secretin (fig.4, middle panel). Adenylate

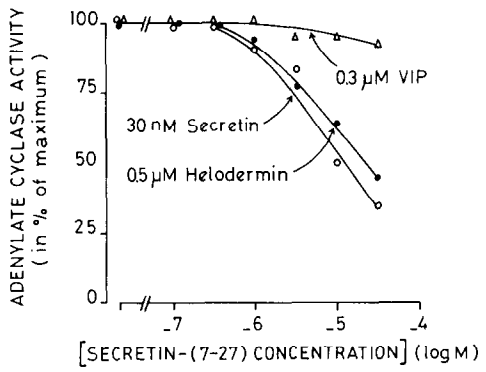

Fig.3. Dose–effect curves of adenylate cyclase inhibition in the presence of increasing concentrations of secretin-(7–27), and in the presence of secretin (○), VIP (△), and helodermin (●). The experiments were performed on rat pancreatic membranes prepared in the absence of 2-mercaptoethanol. The concentrations of secretin (30 nM), VIP (0.3 μM), and helodermin (0.5 μM) used were able to stimulate adenylate cyclase maximally. The results were the mean of 2 experiments and were expressed in % of enzyme activity in the absence of secretin-(7–27).

cyclase activity in rat brain membranes was stimulated by VIP but not by secretin, and helodermin proved to be almost as potent as VIP.

4. DISCUSSION

The crude venom from both *Heloderma suspectum* and *Heloderma horridum* contains a proteolytic-sensitive, thermostable component capable of interacting with VIP receptors in the guinea pig pancreas as it inhibits [^{125}I]VIP binding to pancreatic acini, and stimulates cyclic AMP formation and amylase secretion [14]. We recently presented evidence [13] that the crude venom stimulates adenylate cyclase through secretin-preferring rather than through VIP-preferring receptors, in rat pancreatic plasma membranes. The 5.9-kDa peptide component responsible for this effect has been purified to homogeneity by a method detailed in [1] and has been designated helodermin.

Here, helodermin was shown to share some of

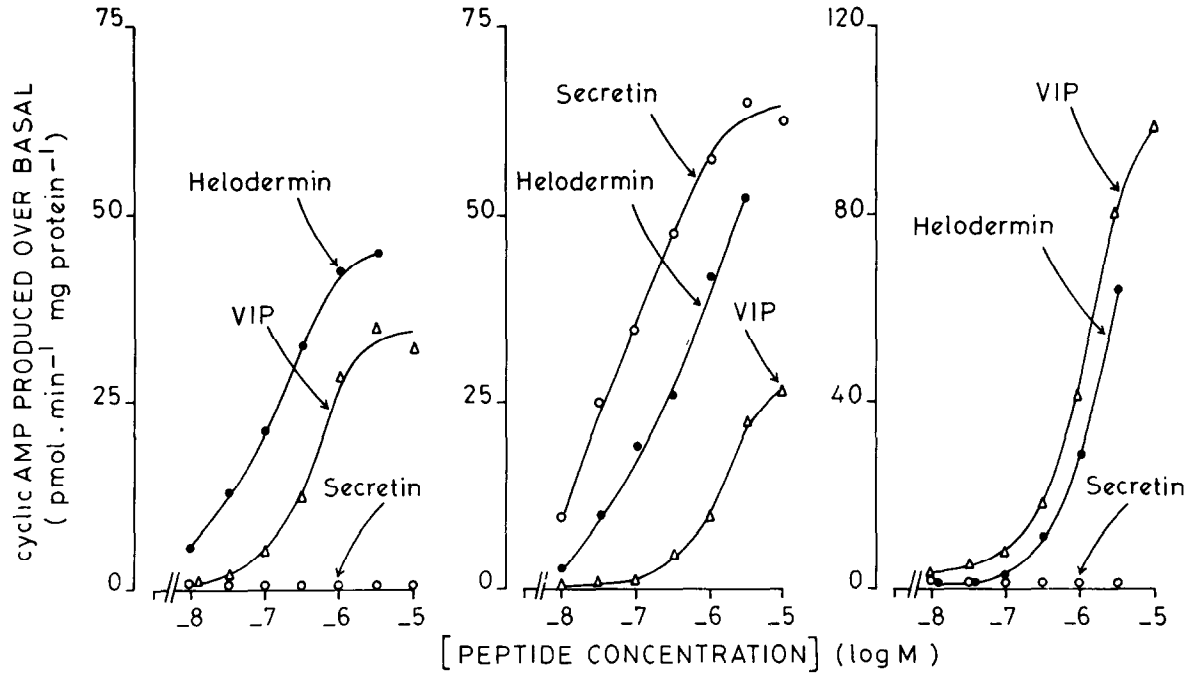

Fig.4. Dose–effect curves of adenylate cyclase activation in presence of secretin (○), VIP (△) and helodermin (●) in human heart membranes (left panel), rat heart membranes (middle panel), and rat brain synaptic membranes (right panel). Data were expressed in pmol cyclic AMP produced · min^{-1} · mg $protein^{-1}$ and were representative of 2 other experiments. Basal activities were, respectively, 22, 31 and 220 pmol cyclic AMP · min^{-1} · mg $protein^{-1}$ for membranes from human heart, rat heart, and rat brain.

the properties of the crude venom: like VIP and secretin, it inhibited [^{125}I]VIP binding and stimulated cyclic AMP accumulation in rat pancreatic acini (fig.1); it also stimulated adenylate cyclase activity in rat pancreatic plasma membranes (fig.2). The affinity of helodermin for rat pancreatic VIP receptors was higher than that of secretin (fig.1, left panel) but its action on rat pancreatic cell membranes appeared, nevertheless, to be mediated through secretin receptors for 3 reasons:

(i) Dose–effect curves of helodermin and secretin on cyclic AMP formation in dispersed acini were comparable (fig.1, right panel);
(ii) Helodermin stimulation of adenylate cyclase activity, like that of secretin and at variance with that of VIP, was shifted only 10-fold to the right in pancreatic membranes prepared in the presence of 2-mercaptoethanol (fig.2);
(iii) Helodermin effects were inhibited by secretin-(7–27), a specific marker of secretin receptors [11,12] (see fig.3).

The fact that helodermin recognized rat pancreatic secretin receptors suggests a structural relationship of helodermin with the pig hormone. This conclusion was supported by the observation that helodermin was as efficient as secretin in stimulating adenylate cyclase activity in rat heart membranes (fig.4, middle panel), a system endowed with secretin-preferring receptors that recognize VIP only poorly [8].

Other helodermin activities were, however, distinct from those of secretin. The new peptide activated adenylate cyclase in rat brain membranes and human heart membranes as potently or even more potently than VIP (fig.4, left and right panels). These two models possess VIP-preferring receptors (with a relatively weak affinity for VIP) and are insensitive to secretin [9,12]. Helodermin was, thus, likely to interact with VIP receptors in these models although the existence of specific helodermin receptors cannot be definitively excluded.

The interaction of helodermin with other receptor systems coupled with adenylate cyclase appeared unlikely: helodermin effects were not inhibited by the β-adrenergic antagonist propranolol on heart membranes, nor by the CCK-antagonist dibutyryl cyclic GMP on pancreatic plasma membranes (not shown). Helodermin differed also from PHI acting on VIP receptors [15]: PHI is, indeed, less potent than VIP on human heart membranes [9] and less potent and efficient than VIP on rat heart membranes [16].

To conclude, helodermin appears to be a peptide related to secretin and VIP but with an original pattern of biological activities. Helodermin interacts with secretin or VIP receptors, depending on the system tested: its behavior is more secretin-like than VIP-like on secretin receptors but displays opposite affinities in the presence of VIP receptors. Of particular pharmacological interest is the fact that helodermin is a potent activator of human cardiac adenylate cyclase so that the peptide may, conceivably, be a better inotropic and chronotropic agent than VIP itself [17].

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REFERENCES

[1] Vandermeers, A., Vandermeers-Piret, M.C., Robberecht, P., Waelbroeck, M., Dehaye, J.P., Winand, J. and Christophe, J. (1984) FEBS Lett. 166, 273–276.
[2] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[3] Dehaye, J.P., Winand, J., Hermans, L., Poloczek, P. and Christophe, J. (1983) Eur. J. Pharmacol. 92, 259–264.
[4] Fraker, P.J. and Speck, J.C. (1978) Biochem. Biophys. Res. Commun. 80, 849–857.
[5] Christophe, J., Conlon, T.P. and Gardner, J.D. (1976) J. Biol. Chem. 251, 4629–4634.
[6] Svoboda, M., Robberecht, P., Camus, J., Deschodt-Lanckman, M. and Christophe, J. (1976) Eur. J. Biochem. 69, 185–193.
[7] Robberecht, P., König, W., Deschodt-Lanckman, M., De Neef, P. and Christophe, J. (1979) Life Sci. 25, 879–884.
[8] Chatelain, P., Robberecht, P., De Neef, P., Deschodt-Lanckman, M., König, W. and Christophe, J. (1980) Pflügers Arch. 389, 21–27.

[9] Taton, G., Chatelain, P., Delhaye, M., Camus, J.C., De Neef, P., Waelbroeck, M., Tatemoto, K., Robberecht, P. and Christophe, J. (1982) Peptides 3, 897–900.
[10] Salomon, Y., Londos, C. and Rodbell, M. (1974) Anal. Biochem. 58, 541–548.
[11] Robberecht, P., Waelbroeck, M., Noyer, M., Chatelain, P., De Neef, P., König, W. and Christophe, J. (1982) Digestion 23, 201–210.
[12] Robberecht, P., Chatelain, P., Waelbroeck, M. and Christophe, J. (1982) in: Vasoactive Intestinal Peptide (Said, S.I. ed) pp.323–332, Raven, New York.
[13] Gillet, L., Robberecht, P., König, W. and Christophe, J. (1983) Regul. Peptides 6, 305.
[14] Raufman, J.P., Jensen, R.T., Sutliff, V.E., Pisano, J.J. and Gardner, J.D. (1982) Am. J. Physiol. 242, G470–G474.
[15] Bataille, D., Gespach, C., Laburthe, M., Amiranoff, B., Tatemoto, K., Vauclin, N., Mutt, V. and Rosselin, G. (1980) FEBS Lett. 114, 240–242.
[16] Christophe, J., Chatelain, P., Waelbroeck, M. and Robberecht, P. (1984) Biomed. Res., in press.
[17] Smitherman, T.C., Sakio, H., Geumei, A.M., Yoshida, T., Oyamada, M. and Said, S.I. (1982) in: Vasoactive Intestinal Peptide (Said, S.I. ed) pp.169–176, Raven, New York.