Specific labelling by [^{125}I]helodermin of high-affinity VIP receptors in rat liver membranes

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Helodermin, a newly isolated peptide from Gila Monster venom, is structurally related to VIP and secretin. When used as radioligand, [^{125}I]helodermin bound rapidly and reversibly to crude rat liver membranes, the dissociation being accelerated by GTP. Competition binding curves of [^{125}I]helodermin and [^{125}I]VIP with unlabelled peptides showed the following order of decreasing affinity: VIP > helodermin > secretin > hpGRF(1–29)-NH_2. The shape of binding curves and of concurrent adenylate cyclase activation is compatible with the specific labelling, by [^{125}I]helodermin, of a class of high-affinity VIP receptors that is capable to stimulate adenylate cyclase.

Gila Monster venom *Helodermin* *Vasoactive intestinal peptide* *Secretin* *Growth hormone releasing factor* *Adenylate cyclase* *Rat liver membrane*

1. INTRODUCTION

Helodermin is a recently purified peptide from the venom of helodermae lizards [1]. On the basis of its biological properties, this peptide is related to VIP, secretin, PHI and hpGRF [2]. The N-terminal amino acid sequence is His-Ser-Asp (like that of VIP and secretin); its amino acid composition is compatible with a 36 amino acid peptide and includes – like that of VIP – two tyrosine residues (personal communication, Dr N. Yanaihara, Shizuoka College of Pharmacy, Japan). Helodermin recognizes specific VIP receptors in rat brain and human heart; it activates adenylate cyclase in rat heart through the occupancy of receptors that are common for secretin and VIP, and can also bind to secretin receptors in acini and membranes from rat pancreas [2,3]. It was of interest, therefore, to test the binding of helodermin to VIP receptors (recognizing secretin with low affinity) in rat liver membranes and the ensuing adenylate cyclase activation.

2. MATERIALS AND METHODS

Helodermin was purified from crude Gila Monster venom as in [1]. A crude preparation of hepatic plasma membranes was obtained from male rats as in [4] and immediately assayed for tracer binding and adenylate cyclase activity.

Helodermin and synthetic VIP (kindly supplied by Dr D.H. Coy, Tulane University, New Orleans, LA) were similarly iodinated by the chloramine-T method as in [5] and purified by cellulose absorption and bovine serum albumin elution as in [6]. The iodination performed on 3 batches of helodermin gave identical results. The specific radioactivity of the tracers was 200–300 μCi/μg.

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Abbreviations: VIP, vasoactive intestinal peptide; GRF, growth hormone releasing factor; PHI, porcine peptide having N-terminal histidine and C-terminal isoleucineamide; hpGRF, human pancreatic growth hormone releasing factor

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Binding studies were conducted by microfuge centrifugation [4]. The radioactivity offered was 70000 and 40000 cpm for, respectively, [^{125}I]helodermin and [^{125}I]VIP. Under all experimental conditions the radioactivity bound was always less than 10% of the radioactivity offered and remained proportional to the amount of membrane protein. Competition curves of unlabelled peptides were not modified by a 3-fold increase in tracer concentration, suggesting that the added unlabelled peptide concentration required for a 50% inhibition of tracer binding was equal to the dissociation constant of the peptide [7].

Adenylate cyclase activity was determined as in [8] as described in [4].

Secretin and hpGRF(1–29)-NH_2 were generous gifts from, respectively, Dr W. König (Hoechst Aktiengesellschaft, Frankfurt/Main) and Dr D.H. Coy (Tulane University, New Orleans, LA).

3. RESULTS

3.1. *Comparative effects of helodermin, VIP, secretin and hpGRF on adenylate cyclase activity in rat liver membranes*

Helodermin and VIP stimulated rat hepatic adenylate cyclase activity to the same maximal value (fig.1); the potency of helodermin (K_{act}, 2 nM) was, however, 7-fold lower than that of VIP (K_{act}, 0.3 nM). Secretin was as efficient as VIP but 200-fold less potent (K_{act}, 60 nM). The apparent maximal efficacy of hpGRF(1–29)-NH_2 was 80% that of VIP and the 3 other peptides and its K_{act} (0.9 μM) was 3000-fold lower than that of VIP.

3.2. *Identification of helodermin binding sites on rat liver membranes*

^{125}I-Labelled helodermin bound rapidly (fig.2) and reversibly (fig.3) to rat liver membranes. Apparent equilibrium was attained after a 30 min incubation at 37°C. Non-specific binding, determined by incubating the tracer and membranes in the presence of 1 μM unlabelled VIP, was low (fig.2). The tracer was stable during the incubation period, based on a 95% precipitability by trichloroacetic acid that did not change significantly after a 30 min incubation (inset, fig.2). The dissociation of the tracer bound was measured after adding 1 μM unlabelled VIP at equilibrium: this dissocia-

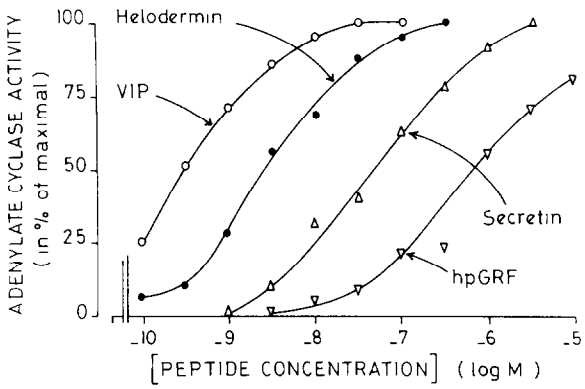

Fig.1. Effects of increasing concentrations of VIP (○), helodermin (●), secretin (△) and hpGRF(1–29)-NH_2 (▽) on adenylate cyclase activity of rat liver membranes. The results were the means of 3 experiments performed in duplicate and were expressed in % of the value observed in the presence of 1 μM VIP. For estimating maximal activity, the basal activity (22.0 ± 1.6 pmol cyclic AMP produced·min^{-1}·mg protein^{-1} was subtracted from the value obtained in the presence of 1 μM VIP (53.2 ± 3.4 ± pmol cyclic AMP produced·min^{-1}·mg protein^{-1}).

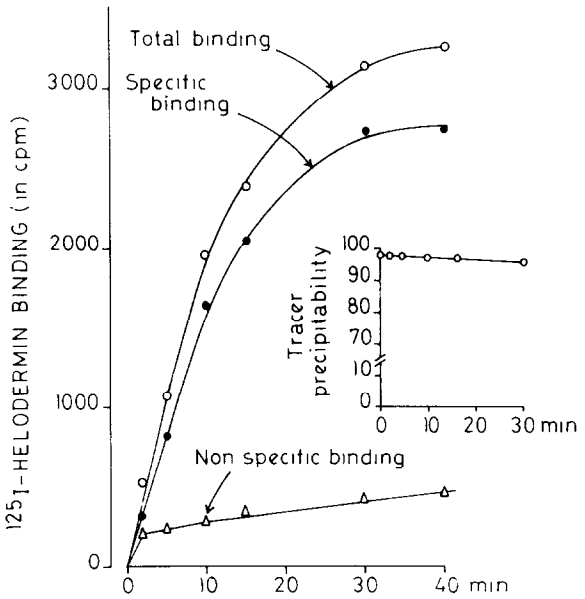

Fig.2. Time course of [^{125}I]helodermin binding to rat liver membranes at 37°C. Specific binding (●) represented the difference between total binding (○) and non-specific binding (△) observed in the presence of 1 μM unlabelled VIP. The inset represents trichloroacetic acid precipitability of the tracer during incubation in the presence of membranes. The data represent 3 experiments.

tion was rapid, 50% of the tracer being released after 5 min at 37°C. Addition of 10 μM GTP markedly accelerated tracer dissociation, 90% being released after 1 min only at 37°C (fig.3).

3.3. *Comparison of helodermin and VIP binding sites*

The specificity of binding sites was studied by comparing competition curves between the two tracers and the 4 reference peptides VIP, helodermin, secretin, and hpGRF(1–29)-NH_2 (fig.4).

[^{125}I]Helodermin and [^{125}I]VIP binding sites recognized the 4 unlabelled peptides. The relative affinities of these peptides were similar, their order of potency remaining in each case: VIP > helodermin > secretin > hpGRF(1–29)-NH_2. Significant differences were, however, apparent when considering the affinities of the unlabelled peptides for binding sites. VIP, helodermin, secretin and hpGRF(1–29)-NH_2 were, respectively, 8-, 25-, 20- and 6-fold more potent in inhibiting [^{125}I]helodermin binding than [^{125}I]VIP binding. Furthermore, competition curves were steeper when performed in the presence of [^{125}I]helodermin than in the presence of [^{125}I]VIP, at least with VIP and helodermin. The concentrations of the 4 peptides provoking a 50% inhibition of [^{125}I]helodermin binding (fig.4, lower panel) were in good agreement with those required for half-maximal adenylate cyclase activation (fig.1).

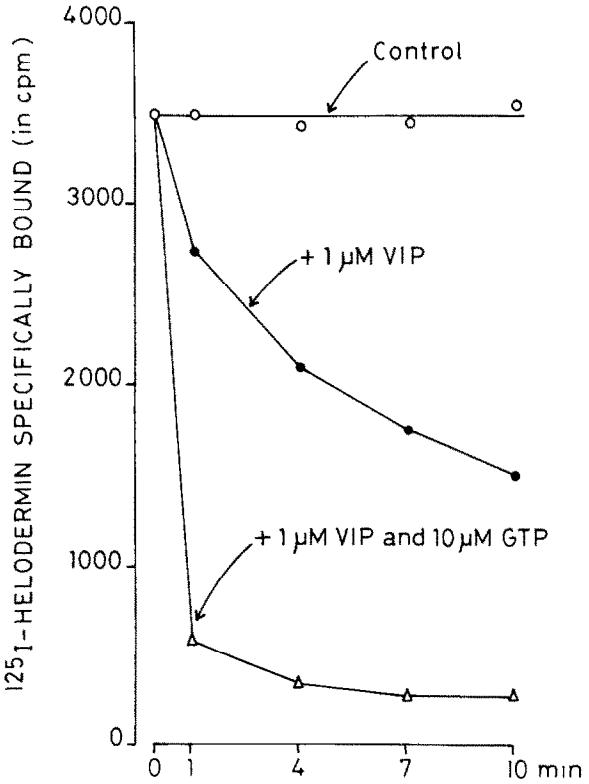

Fig.3. Time course of dissociation of [^{125}I]helodermin bound to rat liver membranes. After a 30 min incubation period of tracer and membranes at 37°C, the radioactivity bound under control conditions (○) was compared to that observed after addition of either 1 μM unlabelled VIP (●) or 1 μM unlabelled VIP and 10 μM GTP (△). The data represent 3 experiments.

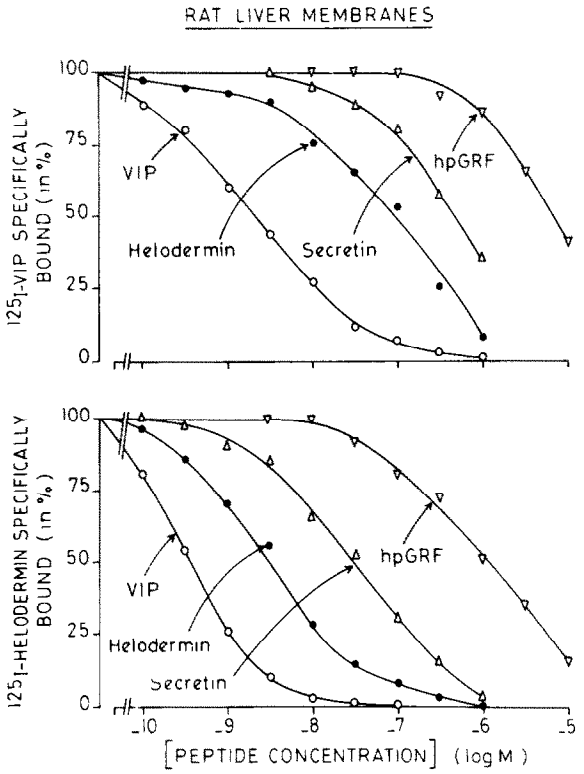

Fig.4. Inhibition of [^{125}I]VIP (upper panel) and [^{125}I]helodermin (lower panel) binding by increasing concentrations of unlabelled VIP (○), helodermin (●), secretin (△) and hpGRF(1–29)-NH_2 (▽). The data are expressed as a percentage of tracer bound in the absence of unlabelled peptide and are the means of 3 experiments performed in duplicate.

4. DISCUSSION

Unlabelled helodermin recognized all binding

sites occupied by [^{125}I]VIP in rat liver membranes (fig.4, upper panel). Its efficacy in activating adenylate cyclase was similar to that of VIP and secretin while its potency was lower than that of VIP but higher than that of secretin (fig.1). These data confirm that helodermin is a peptide structurally related to VIP [2].

VIP receptors in rat liver membranes are heterogeneous and can be resolved into a high-affinity (K_d, 0.8 nM) and a low-affinity (K_d, 9 nM) class of receptors [4]. Based on competition curves, it appears that [^{125}I]helodermin specifically labelled the high-affinity class of VIP binding sites (fig.4). By contrast, the competition curves of unlabelled helodermin and VIP with [^{125}I]VIP were parallel so that [^{125}I]helodermin discriminated the two classes of VIP receptors better than unlabelled helodermin.

[^{125}I]Helodermin may, thus, be a useful tool to study selectively the properties of high-affinity VIP receptors in rat liver membranes. These high-affinity VIP receptors recognized the parent peptides helodermin, secretin and hpGRF and their occupancy, like that of all VIP receptors [9], was markedly reduced by GTP due to an increased dissociation rate of the bound peptide (fig.3). The excellent correlation between binding data and adenylate cyclase activation suggests that the occupancy of these high-affinity sites was responsible for adenylate cyclase activation.

Due to the structural homology of hpGRF with the VIP family of peptides [10–12], hpGRF(1–29)-NH_2 was able to recognize high- as well as low-affinity VIP receptors in rat liver membranes and was capable of activating adenylate cyclase with an efficacy close to that of VIP (figs.1,4). The K_d and K_{act} of hpGRF(1–29)-NH_2 on rat liver membranes were similar to those observed for VIP receptors in membranes from rat intestinal epithelial cells where this peptide is only a weak partial agonist [13].

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