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# Vascular effects of helodermin, helospectin I and helospectin II: a comparison with vasoactive intestinal peptide (VIP)

[1,*]Lars Grundemar & †Edward D. Högestätt

Departments of *Pharmacology and †Clinical Pharmacology, University of Lund, Lund, Sweden

**1** Helodermin, helospectin I and helospectin II, peptides recently isolated from the salivary gland venom of *Heloderma suspectum*, were compared to vasoactive intestinal peptide (VIP) with respect to effects on systemic blood pressure and on isolated femoral arteries in the rat.

**2** They all reduced blood pressure in a dose-dependent manner; helodermin was less effective than VIP. However, at doses higher than 1 nmol $kg^{-1}$ all four peptides reduced blood pressure to about the same extent.

**3** The half-life of the hypotensive effect of VIP was longer than that of helodermin and the helospectins.

**4** VIP and helodermin were equally potent in relaxing femoral arteries precontracted with phenylephrine or prostaglandin $F_{2\alpha}$.

**5** Helospectin I and II relaxed phenylephrine-contracted vessels to the same extent as VIP but with a lower potency.

**6** Addition of VIP 1 $\mu$M to preparations exposed to helodermin 1 $\mu$M or to either of the helospectins did not produce a further relaxation.

**7** The findings indicate that VIP, helodermin and helospectin I and II have a similar profile of action and therefore may act on a common receptor.

## Introduction

Helodermin, helospectin I and helospectin II are 35 aa-, 38 aa- and 37 aa-peptides, respectively, isolated from the salivary gland venom of the lizard *Heloderma suspectum* (Hoshino *et al.*, 1984; Parker *et al.*, 1984). They are all structurally similar to the neuropeptide vasoactive intestinal peptide (VIP) (Table 1). Some biological actions of helodermin resemble those of VIP. Both peptides activate adenylate cyclase in plasma membranes of the rat pancreas (Vandermeers *et al.*, 1984) but only helodermin induces amylase secretion (Konturek *et al.*, 1989). Helodermin-like peptides occur in high concentrations in the thyroid C cells and in the noradrenaline containing cells of the adrenal medulla in many mammals (Sundler *et al.*, 1988; Grunditz *et al.*, 1989; Bjartell *et al.*, 1989). In the dog, intra-arterial infusion of helodermin, like VIP, causes a dose-dependent increase in femoral blood flow and intravenous injection produces systemic hypotension and tachycardia (Naruse *et al.*, 1986; Konturek *et al.*, 1989). In the present study, vascular effects of helodermin and the helospectins in the rat were compared to those of VIP on systemic blood pressure and on isolated distal femoral arteries.

[1] Author for correspondence at Department of Pharmacology, University of Lund, Sölvegatan 10, S-223 62 Lund, Sweden.

## Methods

### *Animals*

Sprague-Dawley rats of either sex, weighing 250–300 g, were used in all experiments.

### In vivo *experiments*

Rats were anaesthetized by intraperitoneal administration of 0.5 ml ketamine (50 mg $ml^{-1}$) (Ketalar, Parke & Davis) and 0.2 ml xylazin (20 mg $ml^{-1}$) (Rompun, Bayer). The left jugular vein and right femoral artery were cannulated with polyethene catheters for administration of the peptides and for continuous recording of systemic blood pressure via a Statham P23 pressure transducer, connected to a Grass model 7 polygraph. Experimentation was started when the blood pressure had stabilized at 110–130 mmHg. The peptides were infused slowly in a volume of 100 $\mu$l, followed by washing the catheter with 100 $\mu$l saline.

### In vitro *experiments*

Rats were killed by decapitation. The distal part of the femoral artery (0.2 mm diameter) was removed and cut into

**Table 1** Comparison of the amino acid sequence of vasoactive intestinal peptide (VIP) with those of helodermin and the helospectins

VIP (human, porcine)

1 5 10 15 20 25

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 (*Heloderma suspectum*)

1 5 10 15 20 25 30 35

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 + (II) (*Heloderma suspectum*)

1 5 10 15 20 25 30 35

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)-*

The last amino acid in parentheses belongs to the helospectin sequence. Identical residues are underlined. * = $NH_2$.

1–2 mm long segments. The preparations were transferred to a thermostated bath (37°C), containing Krebs solution of the following composition (in mM): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 15.0, $NaH_2PO_4$ 1.2 and glucose 6.0. The solution was bubbled continuously with 95% $O_2$ and 5% $CO_2$, giving a pH of 7.4. The vascular segments were mounted on two L-shaped stainless steel wires (0.1 mm diameter), one of which was connected to a force-displacement transducer (Grass Instrument FT 03C) for continuous tension recording on a Grass polygraph (Högestätt *et al.*, 1983). The resting tension was gradually adjusted to 2–4 mN. The contractile capacity was examined by adding an isotonic 60 mM potassium Krebs solution (NaCl replaced by KCl); these contractions served as an internal control. Concentration-response curves were constructed for each drug; drugs were added in a cumulative manner. Relaxation was studied in preparations precontracted by phenylephrine (3 $\mu$M) or prostaglandin $F_{2\alpha}$ (3–10 $\mu$M). These concentrations induced contractions that were 60–80% of the maximum response for each agent.

### *Statistics*

$pEC_{50}$ values for the peptides were determined graphically by linear interpolation. The results are expressed as mean values ± s.e.mean; *n* refers to the number of animals or arteries examined. Statistical analysis of the results was performed by means of Student's *t* test and by the two way analysis of variance. Statistical significance was accepted when $P < 0.05$.

### *Drugs*

Phenylephrine hydrochloride (PhE) (Sigma, U.S.A.), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Amoglandin, Astra, Sweden) were provided in ampoules. VIP, helodermin, helospectin I and helospectin II were obtained from Peninsula, Belmont, CA, U.S.A. The drugs were dissolved in and/or diluted with 0.9% saline.

## Results

### In vivo *experiments*

Like VIP, helodermin, helospectin I and II reduced blood pressure in a dose-dependent manner (Figure 1). Helodermin was less effective than VIP in the low dose range. At doses higher than 1 nmol $kg^{-1}$, the blood pressure was reduced to about the same extent by all four peptides. The half-life of the hypotensive effect, expressed as the interval between injection and the time when 50% of the effect remained, was longer for VIP than for the other peptides (Figure 2a).

### In vitro *experiments*

PhE and $PGF_{2\alpha}$ induced concentration-dependent contractions of similar potency. The $pEC_{50}$ values (denoting the negative logarithm of the drug concentration (M) inducing half maximum contraction) for $PGF_{2\alpha}$ ($n = 5$) and PhE ($n = 5$) were 5.52 ± 0.03 and 5.61 ± 0.06 and the corresponding $E_{max}$ values (denoting the maximum contraction for each drug) were 11.0 ± 1.4 mN and 14.0 ± 0.9 mN, respectively. VIP and helodermin relaxed the precontracted arterial segments with similar potency (Figure 3, Table 2). However, the maximum relaxation evoked by helodermin was less than that evoked by

**Table 2** Relaxation by vasoactive intestinal peptide (VIP), helodermin, helospectin I and helospectin II of phenylephrine-contracted rat femoral arteries

| | n | $pEC_{50}$ | $E_{max}$ |
|---|---|---|---|
| VIP | 10 | 7.32 ± 0.09 | 70 ± 4 |
| Helodermin | 7 | 7.53 ± 0.07 | 51 ± 7* |
| Helospectin I | 8 | 6.82 ± 0.13* | 72 ± 4 |
| Helospectin II | 6 | 6.93 ± 0.05** | 68 ± 4 |

$pEC_{50}$ denotes the negative logarithm of the peptide concentration (M) producing half maximum relaxation. $E_{max}$ denotes the maximum relaxation in %. Asterisks indicate statistical significance for differences between VIP and the other peptides; *$P < 0.05$; **$P < 0.01$.

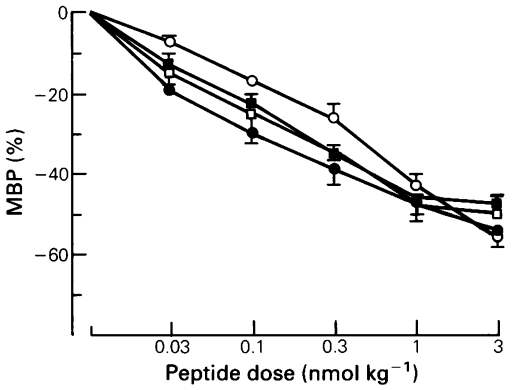


**Figure 1** Dose-response curves for the hypotensive effect of vasoactive intestional peptide (VIP, ●, $n = 5$), helodermin (○, $n = 5$), helospectin I (■, $n = 6$) and helospectin II (□, $n = 5$) in anaesthetized rats. Mean blood pressure (MBP) before injection of peptides was 120 ± 2.4 mmHg, ($n = 21$). By using the two way analysis of variance, it was observed that the dose-response curves to VIP and helodermin were significantly different, $P < 0.001$.

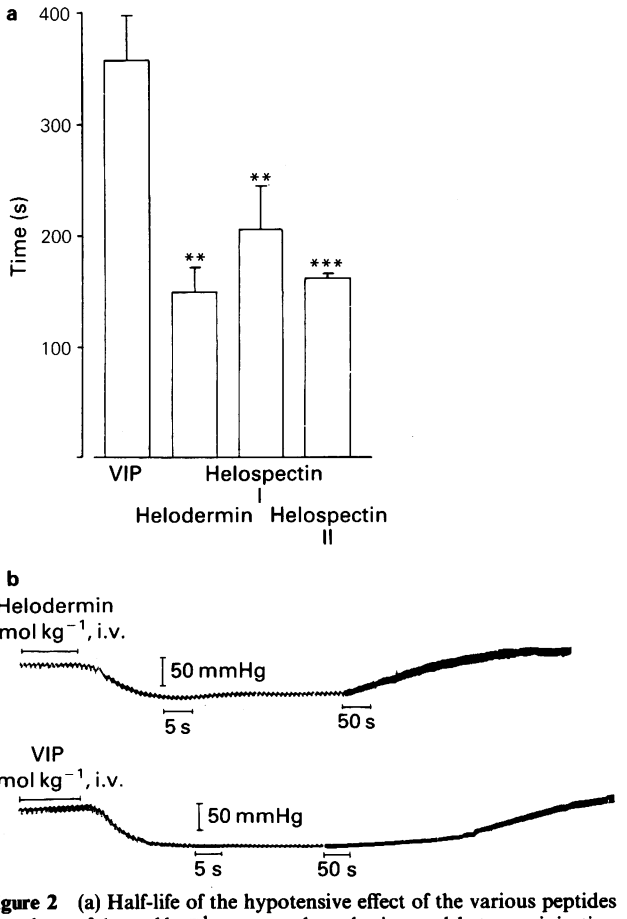


**Figure 2** (a) Half-life of the hypotensive effect of the various peptides at a dose of 1 nmol $kg^{-1}$, expressed as the interval between injection and the time when 50% of the hypotensive effect remained. The statistical significance for the difference between the effects of vasoactive intestional peptide (VIP) and those of the other peptides is indicated: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. (b) The effects of i.v. injections of VIP and helodermin on continuous recordings of rat systemic blood pressure.

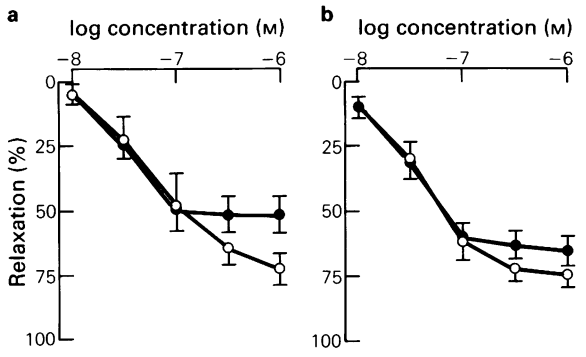


**Figure 3** Relaxation by vasoactive intestinal peptide (VIP, ○, $n = 5$) and helodermin (●, $n = 6$) of femoral arteries precontracted with 3 $\mu$M phenylephrine (PhE, a) or 3–10 $\mu$M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, b). Relaxation is expressed as a percentage of the contraction elicited by PhE or $PGF_{2\alpha}$.

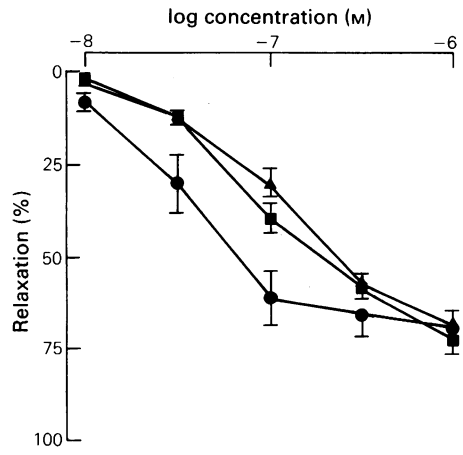


**Figure 4** Relaxation by vasoactive intestinal peptide (●), helospectin I (■) and helospectin II (▲) of femoral arteries precontracted with phenylephrine 3 $\mu$M. For details, see Figure 3.

VIP in PhE-contracted arteries, but very similar to that evoked by VIP in $PGF_{2\alpha}$-contracted vessels. Helospectin I and II relaxed PhE-contracted vessels to the same extent as VIP, but with a lower potency (Figure 4, Table 2).

Addition of VIP 1 $\mu$M to preparations maximally relaxed by helodermin 1 $\mu$M ($n = 12$) or *vice versa* ($n = 6$) did not produce a further relaxation (data not shown).

Similarly, addition of VIP 1 $\mu$M to preparations exposed to 1 $\mu$M of each of the helospectins ($n = 9$–13) or *vice versa* ($n = 2$) did not produce a further relaxation (data not shown).

## Discussion

The results show that helodermin, helospectin I and helospectin II, reduce blood pressure in the rat much like VIP, but that helodermin is less effective than VIP in the low dose-range. VIP had a longer duration of action than the other three peptides. Whether this reflects different susceptibility to the proteolytic activity of rat plasma, or different affinity for a common receptor remains to be established. In contrast, helodermin produces a more long-lasting increase in canine femoral blood flow than VIP (Naruse *et al.*, 1986).

In accordance with the vasodepressor effect, all four peptides were found to relax precontracted femoral arteries, although the helospectins were less potent than VIP and helodermin. However, the maximum relaxation induced by the four peptides was of similar magnitude. Further addition of any of the other peptides did not produce a further relaxation.

Helodermin and the helospectins display structural homology with VIP in 15 residues (Table 1). The findings suggest that VIP, helodermin and the helospectins have similar vascular effects. In rat liver membranes, helodermin is known to bind with high affinity to VIP receptors (Robberecht *et al.*, 1984). It is therefore not inconceivable that helodermin and VIP act on a common receptor type in the vascular bed. The helospectins may also act by the same mechanism.

The physiological relevance of the vascular effects of helodermin and helospectins is unclear. The existence of helodermin-like peptides in mammals, notably in the adrenal medulla (Bjartell *et al.*, 1989) may suggest that they could play a role in cardiovascular regulation, provided that the mammalian homologue of lizard helodermin has the same bioactivity profile.

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