Glibenclamide-Sensitive Hypotension Produced by Helodermin Assessed in the Rat

Naotsugu HoriKawa,a Kazuyoshi Kataha,a Nobue WatAnab,e Kunio Ishii,a,1 Noboru Yanaihara,b Yoshihio Tanaka,b,∗x2) Koki Shigenobuc and Koichi Nakayamaa

Department of Pharmacologya and Laboratory of Bioorganic Chemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52–1 Yada, Shizuoka-shi, Shizuoka 422–8526, Japan and Department of Pharmacology, Toho University School of Pharmaceutical Sciences, 2–2–1 Miyama, Funabashi-shi, Chiba 274–8510, Japan.

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The effects of helodermin, a basic 35-amino acid peptide isolated from the venom of a lizard salivary gland, on arterial blood pressure and heart rate were examined in the rat, focusing on the possibility that activation of ATP-sensitive K+ (KATP) channels is involved in the responses. The results were also compared with those of vasoactive intestinal polypeptide (VIP). Helodermin produced hypotension in a dose-dependent manner with approximately similar potency and duration to VIP. Hypotension induced by both peptides was significantly attenuated by glibenclamide, which abolished a levromakalin-produced decrease in arterial blood pressure. Oxygenmoglobin did not affect helodermin-induced hypotension, whereas it shortened the duration of acetylcholine (ACh)-produced hypotension. These findings suggest that helodermin-produced hypotension is partly attributable to the activation of glibenclamide-sensitive K+ (KATP) channels, which presumably exist on arterial smooth muscle cells. EDRF (endothelium-derived relaxing factor/nitric oxide does not seem to play an important role in the peptide-produced hypotension.

Key words helodermin; vasoactive intestinal polypeptide (VIP); glibenclamide; ATP sensitive K+ channel

Helodermin, a basic 35-amino acid peptide which was originally isolated from the venom of the salivary gland of the Gila monster,3) has a chemical structure close to secretin and vasoactive intestinal polypeptide (VIP).4) Although helodermin was first demonstrated to exist in an animal other than a mammal or a bird as a secretin/VIP related peptide, it also exists in high density in many mammalian tissues, such as brain, gut, salivary glands,5) thyroid parafollicular (C) cells,6) and noradrenergic cells of adrenal medulla7) in rodents such as mice and rats. As expected from its structural similarity to VIP, helodermin was shown to exert biological and pharmacological actions similar to VIP in many biological systems, such as the cardiovascular system, including heart and blood vessels.8–11)

In the cardiovascular system, helodermin has been reported to cause systemic hypotension, tachycardia and an increase in femoral blood flow in various animal species, such as dogs8) and rats,10) and to effect relaxation of isolated rat femoral10) and mesenteric11) arteries. Since VIP was demonstrated to relax arteries by activating ATP-sensitive K+ (KATP) channels,12) the possibility was raised that activation of these channels is involved in the action of helodermin in vivo. The role of endothelium, with particular reference to the possible participation of endothelium-derived relaxing factor (EDRF)/nitric oxide (NO), was also examined in helodermin-produced hypotension. The results were compared with those of VIP.

MATERIALS AND METHODS

Measurement of Systemic Blood Pressure (BP) and Heart Rate (HR) Male Wistar rats (SLC, Hamamatsu, Japan) at 8–10 weeks old, weighing about 250 g, were anesthetized with α-chloralose (80 mg/kg) and urethane (0.8 g/kg) given intraperitoneally. A left carotid artery was cannulated for the measurement of direct continuous systemic arterial BP with a pressure transducer (Model TP-200T, Nihon Kohden, Tokyo, Japan) through a carrier amplifier (Model EF-601G, Nihon Kohden, Tokyo, Japan). Mean arterial BP was derived on-line from the phasic signals using a 2-Hz filter. HR was monitored with a HR counter (Model AT-601G, Nihon Kohden, Tokyo, Japan) triggered by arterial pressure pulse. Both BP and HR were recorded on a pen-writing recorder (Model R-62, Rikadenki, Tokyo, Japan). A cannula was also placed in the left external jugular vein for administration of drugs (i.v.). The animals were allowed to spontaneously breathe through a tracheal cannula. Experiments were started when BP and HR had stabilized. The peptides were injected slowly in a volume of less than 50 μl per 100 g body weight and followed by washing the catheter with 100 μl saline.

Drugs The drugs used in the present study were as follows: helodermin and VIP were synthesized by one of the authors (N.Y.). Glibenclamide, urethane, atropine sulfate and dl-propranolol hydrochloride (Sigma, St. Louis, M.O., U.S.A.), α-chloralose (Tokyo Kasei, Tokyo, Japan), acetylcholine chloride (ACH, Daiichi, Tokyo, Japan) were commercially available. Levromakalin was donated by Beecham Japan (Tokyo, Japan). Helodermin and VIP were dissolved in 0.01 N HCl at a concentration of 300 μM and diluted with physiological salt solution to the desired concentrations. Glibenclamide was dissolved in 0.1 N NaOH at a concentration of 50 μM and diluted with 5% glucose to 10 mM. Levromakalin was dissolved in 70% ethanol at a concentration of 10 mM. Both were then diluted to the desired concentrations with saline. All other drugs were dissolved and diluted in with saline. Oxygenmoglobin was prepared by the method by Martin et al.13)

Statistical Analysis Results are expressed as mean values±S.E.M. Statistical analysis was made by use of Student's paired or unpaired t test. p values less than 0.05 were considered significant.

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RESULTS

Effects of Helodermin on Systemic BP and HR. The systolic and diastolic BP and the HR of the control rats used in the present study were 113.9±3.0 mmHg and 80.5±2.9 mmHg, and 373±12 beats/min (n = 21), respectively.

Figure 1 shows the effects of helodermin and VIP on systemic BP and HR in anesthetized rats. Helodermin (1.5 nmol/kg i.v.) lowered arterial BP by about 30% (30 mmHg) from the basal level, without any prominent effects on the HR (Fig. 1A, left panel). VIP (1.5 nmol/kg i.v.) elicited an almost similar hypotensive action (about 30%, 30 mmHg) (Fig. 1A, right panel). The duration of hypotensive actions produced by these peptides were similar. These hypotensive responses were reproducible (data not shown). Helodermin- and VIP-induced hypotension in the rat was not affected by atropine (2 mg/kg i.v.) or propranolol (0.5 mg/kg i.v.) (data not shown), suggesting that muscarinic-ACh and β-adrenergic receptors are not involved in the hypotension produced by these two peptides. Arterial BP and HR were not significantly modified by i.v. infusion of the vehicle.

The summarized results obtained from i.v. infusion of helodermin (Fig. 1B, left panel, open circles) and VIP (Fig. 1B, left panel, filled circles) showed that both peptides decreased arterial BP in a dose-dependent manner. Helodermin and VIP showed very similar mean arterial BP decreases at doses from 0.15 to 50 nmol/kg, except at 5 nmol/kg. Fifty nmol/kg was the maximal dose of helodermin that we could test in the present study. The tentative ED50 values, which are the doses to produce 50% reduction in BP, compared to the maximal changes (helodermin (2.2±0.7 nmol/kg, n = 7) and VIP (1.1±0.2 nmol/kg, n = 7)), were not significantly different from each other. These peptides exhibited similar duration of hypotensive action, expressed as the half recovery time (Fig. 1B, right panel). For example, helodermin and VIP at 1.5 nmol/kg (similar to the ED50 value of both peptides) decreased arterial BP by 25.5±4.4 mmHg with a half recovery time of 1.4±0.3 min (each, n = 6), and 29.4±2.3 mmHg with a recovery time of 1.8±0.4 min (each, n = 6), respectively. They were not significantly different from each other.

Effects of Glibenclamide on the Hypotensive Effects of Helodermin and VIP. Figure 2 shows the effect of glibenclamide on the hypotensive actions produced by helodermin and VIP. Glibenclamide (20 mg/kg i.v.) was administered to the rat 20 min before the administration of helodermin (1.5 nmol/kg i.v.) or VIP (1.5 nmol/kg i.v.). The injection of glibenclamide (20 mg/kg i.v.) per se changed mean arterial BP from the basal value of 95.7±10.3 mmHg to 114.1±6.4 mmHg, but they were not statistically significant (each n = 5, p > 0.05). After administration of glibenclamide (20 mg/kg i.v.), the hypotension produced by helodermin (1.5 nmol/kg i.v.) (Fig. 2A, B) or VIP (1.5 nmol/kg i.v.) (Fig. 2B) was significantly reduced (p < 0.01). In contrast, the half recovery times of hypotension for both peptides were not significantly affected by treatment with glibenclamide (Fig. 2C). The hypotension (31.2±4.4 mmHg, n = 3) induced by levromakalim (105 nmol/kg i.v.), a K ATP channel opener, was abolished by treatment with glibenclamide (20 mg/kg i.v.).

Effects of Oxyhemoglobin on the Hypotensive Effects of Helodermin and VIP. Oxyhemoglobin (65 mg/kg i.v.) was administered to the rat 20 min before i.v. injection of heloder-
Table 1. Effects of Oxyhemoglobin (oxyHb) on Hypotensive Responses to Helodernin (Hd), VIP and ACh, and Their Half Recovery Times (T₁/₂).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>+oxyHb (65 mg/kg)</th>
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<tr>
<td></td>
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<td>(65 mg/kg)</td>
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<tr>
<td>ΔMAPB (mmHg)</td>
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<tr>
<td>Hd</td>
<td>23.3±3.9</td>
<td>24.6±6.6</td>
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<tr>
<td>(1.5 nmol/kg)</td>
<td>(1.5 nmol/kg)</td>
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<tr>
<td>VIP</td>
<td>40.5±8.1</td>
<td>46.3±8.7</td>
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<tr>
<td>(1.5 nmol/kg)</td>
<td>(16.5 nmol/kg)</td>
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<tr>
<td>ACh</td>
<td>39.2±5.9</td>
<td>42.2±5.6</td>
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<tr>
<td>(16.5 nmol/kg)</td>
<td>(16.5 nmol/kg)</td>
<td></td>
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<tr>
<td>T₁/₂ (s)</td>
<td></td>
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<tr>
<td>Hd</td>
<td>114.5±26.0</td>
<td>108.5±18.0</td>
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<tr>
<td>(1.5 nmol/kg)</td>
<td>(1.5 nmol/kg)</td>
<td></td>
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<tr>
<td>VIP</td>
<td>231.0±68.3</td>
<td>298.5±107.3</td>
</tr>
<tr>
<td>(1.5 nmol/kg)</td>
<td>(16.5 nmol/kg)</td>
<td></td>
</tr>
<tr>
<td>ACh</td>
<td>45.7±9.2</td>
<td>20.8±2.7*</td>
</tr>
<tr>
<td>(16.5 nmol/kg)</td>
<td></td>
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Each value represents the mean values±S.E.M. of 6 experiments. The difference from the corresponding control response is statistically significant (*p<0.05). MAPB: mean arterial blood pressure.

min (1.5 nmol/kg), VIP (1.5 nmol/kg), or ACh (16.5 nmol/kg). Oxyhemoglobin significantly increased mean arterial BP from 90.1±11.3 mmHg to 100.8±10.7 mmHg (p<0.05, each n=6), but did not appreciably affect the HR (before: 340.6±34.5 beats/min vs. after: 344.0±37.8 beats/min, each n=6). As summarized in Table 1, the hypotensive action and half recovery times for helodernin and VIP were not affected by oxyhemoglobin. In contrast, the duration of hypotension induced by ACh (16.5 nmol/kg), which was expressed as the half recovery time, was significantly shortened by oxyhemoglobin (before: 45.7±9.2 s vs. after: 20.8±2.7 s, each n=6, p<0.05), though the mean arterial BP decrease produced by ACh was not affected by treatment with oxyhemoglobin.

DISCUSSION

The present findings showed that helodernin was approximately equipotent with VIP in inducing hypotension in the rat.

The duration of hypotensive action produced by helodernin was similar to that of VIP in the rat, however, helodernin-induced relaxation of mesenteric artery isolated from rats lasted longer than the VIP-induced response.11 Naruse et al.13 also reported that helodernin, when administered intratranerally, increased femoral blood flow for a longer time than VIP. Helodernin was shown to recognize, or bind to, the receptor that specifically recognizes VIP in rat pancreatic acini4 and rat liver.5 Thus, it is conceivable that helodernin also acts on the same receptor that VIP specifically recognizes in arterial smooth muscle cells in rats, which is then followed by vascular relaxation. If helodernin binds to authentic VIP receptors, the C-terminal 7-residue peptide extension of helodernin may be responsible for the longer action of this peptide.16 In fact, Hd[1-27]NH₂ was shown to exhibit a somewhat lower vasodilating potency with shorter duration of action, suggesting that the C-terminal part of helodernin, containing the characteristic Pro-Pro-Pro sequence, stabilizes the active core of helodernin.9 However, when administered intravenously, as in the present study, the C-terminal part of helodernin might be degraded by proteases in the plasma of the rat, and this may explain why the hypotensive duration of helodernin and VIP were not significantly different from each other, in spite of the longer vasoconstricting action of helodernin in the isolated mesenteric artery. The lack of prominent heart rate increases (reflex tachycardia) following hypotension produced by helodernin and VIP, might be attributable to the anesthetics (α-chloralose plus urethane) used in the present study, since hypotension induced by Ca²⁺ channel blockers (nifedipine, nicardipine, etc.) are also not associated with a pronounced heart rate increase under the same experimental conditions (unpublished observations).

It is unlikely that EDRF/NO plays an important role in helodernin- or VIP-induced hypotension in rats because oxyhemoglobin, an inactivator of EDRF/NO,13 did not affect the hypotensive actions produced by these peptides, whereas it shortened the duration of ACh-induced hypotension. On the other hand, helodernin- and VIP-induced hypotension was partly, but significantly inhibited by glibenclamide, which abolished levromakalin-induced hypotension. Thus, the hypotensive action of these peptides may be partly mediated by glibenclamide-sensitive K⁺ (KATP) channels. KATP channels have been shown to play important roles in membrane hyperpolarization and vascular relaxation produced by VIP in rabbit cerebral artery.13 Our present findings suggest that the activation of KATP channels, which presumably exist at the level of peripheral resistance arteries, significantly contribute to the hypotension produced not only by VIP, but also by helodernin. Helodernin could play a role in cardiovascular regulation, since helodernin-like peptides are known to exist in mammals, notably in the adrenal medulla.7 Further studies are required to settle the physiological role of helodernin in the circulatory system of mammals, and to elucidate the intracellular mechanisms underlying the activation of KATP channels after stimulation of helodernin/VIP receptors.

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REFERENCES AND NOTES

1) Present address: Department of Molecular Pharmacology, Kitasato University School of Pharmaceutical Sciences, 9–1 Shirokanedai-5, Minato-ku, Tokyo 108–8641, Japan.
2) Present address: Department of Pharmacology, Toho University School of Pharmaceutical Sciences, 2–2–1 Miyama, Funabashi-shi, Chiba 274–8510, Japan.