Vasoactive Intestinal Polypeptide (VIP) Stimulates Fibrinolytic System in the Rat

Masaki HAGIWARA, Kazuya HONGO and Tadanori MORIKAWA
Fuji Chemical Industries, Ltd., 530 Chokeiji, Takaoka, Toyama 933, Japan
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Abstract—We have studied the fibrinolytic effect of VIP in rats. Intravenous injection of VIP enhanced blood fibrinolytic activity in a dose-related manner. The euglobulin fraction obtained from intact rat plasma incubated with VIP did not produce an increase in fibrinolytic activity, while dextran sulfate (DS) and urokinase (UK) showed the activity. VIP solution placed on a plasminogen-rich fibrin plate did not show fibrinolysis. VIP had neither a plasminogen activator nor plasmin activity. VIP may release plasminogen activators into the blood.

VIP was first isolated from porcine duodenum and originally considered to be the gut hormone involved in smooth muscle relaxation and vasodilation (1). VIP has wide-spread distribution, occurring in neurons of both the central and peripheral nervous systems (2, 3). The peptide is released by a variety of short-term biological responses (4). This peptide has been studied for its many pharmacological actions, but influences of VIP on the blood fibrinolytic system is as yet unclear. The object of the present study was to focus on the pharmacological action of VIP in fibrinolysis. Recently, Liu et al. have reported that VIP stimulated the tissue-type plasminogen activator (t-PA) activity using cultured granulosa cells (5). The t-PA for hemostasis is released from the vascular wall into the circulating blood (6). These could indicate that VIP can cause blood fibrinolysis in the body. However, several studies indicate that the mode of VIP action varies from tissue to tissue. In this paper, we report whether VIP can cause blood fibrinolysis in vivo in the rat.

Male Wistar rats (ST, Sankyo Lab.), weighing 190–260 g, were used. Animals were anesthetized with urethane (1.25 g/kg, i.p.). The jugular vein was exposed for blood collection. Blood was removed with a plastic syringe containing 1/10 volume of sodium citrate (3.8%). Approximately 0.5 ml each of blood was withdrawn at 10 min before the drug injection and at 1, 3, 10 and 30 min after. Drugs dissolved in saline solution, except for VIP (0.001 M acetic acid solution containing 0.01% BSA), were injected into the femoral vein. Control animals were given saline or the vehicle for VIP.

Fibrinolytic activity in the blood was measured by the modified method reported by Gallimore et al. (7). A 200-μl portion of plasma was added to 3.8 ml of ice-cold water; the pH was then brought to 5.65±0.05 by addition of 80 μl of 1% acetic acid. After the diluted plasma was kept at 4°C for 30 min, the precipitated euglobulin fraction (EF) was collected by centrifugation for 5 min at 4°C. The euglobulin precipitate was dissolved in 200 μl of Tris HCl buffer (50 mM, pH 7.4). In another experiment, 20 μl of drug solution was added to intact plasma (200 μl) before the pH adjustment, and the plasma was kept at room temperature for 30 min. EFs were then obtained as described above. Fibrin plates were prepared as follows: 0.5% solution of plasminogen-rich bovine fibrinogen dissolved in 100 mM borate saline buffer (pH 7.8) was filtered, and 740 μl of 10 U/ml human thrombin dissolved in 80 mM CaCl₂ was mixed with 20 ml of the filtrated fibrinogen solution. The mixture was spread over the surface of a plastic Petri dish immediately after mixing, and the fibrin film was allowed to form at room temperature. Duplicate 10-μl samples of EFs were applied.
to the fibrin plate. In other experiments, 10 µl of solution containing VIP or the other drugs were introduced into the fibrin plate. The plate was incubated at 37°C for 20 hr. The diameters of the lysis area were measured, and the activity was expressed as the area (short X long diameter, mm²). Values represent the mean±S.E. The same lots of bovine fibrinogen were used for all experiments.

The following chemicals were used: VIP (Peptide Institute, Inc., Osaka), dextran sulfate sodium (MW ca. 5000, Sigma Chemicals, St. Louis, MO), urokinase, human thrombin (Midori-Juji, Osaka), bovine fibrinogen, and bovine plasmin (Sigma).

VIP, dextran sulfate (DS) and urokinase (UK) increased the euglobulin lysis area in the plasma collected 1 min after the injection, in a dose-related manner (Table 1). The effect of VIP at 10⁻⁸ mol/kg was evident at 1, 3 and 10 min after the injection. The activity was more prolonged than that of UK, but was shorter than that of DS. While the peak of UK's activity was remarkable, those of VIP and DS were not so clear as UK. VIP reduced the blood pressure, but DS did not in the rat (data not shown). Repeated collection of blood may influence blood pressure. However, the control values were almost constant during the experiment. Therefore, repeated collection of blood may not remarkably influence the activity of these compounds.

In the in vitro experiments, EF obtained from intact rat plasma in the presence of VIP did not increase the euglobulin lysis area in the dose range of 3×10⁻⁹ to 10⁻⁸ M, whereas DS (10⁻⁷ to 2×10⁻⁶ M) and UK (1.2 U/ml and 2.5 U/ml) increased the euglobulin lysis area in a concentration-related manner (Table 2). VIP and DS solution placed on fibrin plates failed to produce measurable lysis. However, UK on the fibrin plate showed lysis: 10.2±1.1, 37.3±5.0 and 60.5±4.4 mm² (n=4) in concentrations of 0.5, 1 and 2 U/ml, respectively. Bovine plasmin also produced activity: 62.4±5.5 mm² (n=4) at 0.25 U/ml.

An intravenous injection of VIP enhanced plasma fibrinolytic activity in the rat. However, EF of the plasma that was treated with VIP in vitro did not enhance fibrinolysis, while DS had fibrinolytic activity. DS potentiates the interaction between factor XII, prekallikrein and high molecular weight kininogen, producing activated factor XII (F XIIa) and kallikrein (8, 9). F XIIa has been shown to enhance the fibrinolytic activity of plasma euglobulin (10). Therefore, it is unlikely that VIP stimulates the intrinsic fibrinolytic system.

### Table 1. Fibrinolytic activity in vivo of VIP, DS and UK

| Compounds | Dose | N | Time (min) after the administration |
|-----------|------|---|----------------------------------|
|           | mol/kg |   | 0 | 1 | 3 | 10 | 30 |
| Control   | 5     | 14.9 | 19.7 | 18.4 | 19.2 | 16.7 |
|           | ± 2.9 |      | ± 4.3 | ± 5.2 | ± 4.4 | ± 3.4 |
| VIP       | 3×10⁻⁸ | 3 | 17.8 | 29.2* | 25.1* | 20.8 | 16.7 |
|           | ± 2.2 |      | ± 4.1 | ± 3.8 | ± 2.8 | ± 2.7 |
|           | 10⁻⁸  | 4 | 15.6 | 32.7** | 30.2** | 28.2** | 18.3 |
|           | ± 1.5 |      | ± 2.3 | ± 1.7 | ± 0.6 | ± 2.7 |
| DS        | 10⁻⁶  | 4 | 20.6 | 72.2** | 76.4** | 70.2** | 56.7** |
|           | ± 1.7 |      | ± 2.5 | ± 2.1 | ± 4.9 | ± 2.0 |
|           | 2×10⁻⁶ | 4 | 17.8 | 76.8** | 92.4** | 96.5** | 73.7** |
|           | ± 4.3 |      | ± 5.0 | ± 5.3 | ± 2.4 | ± 7.3 |
| UK        | 1250 U/kg | 3 | 13.9 | 111.7* | 60.7* | 28.0 | 18.5 |
|           | ± 1.4 |      | ±27.0 | ±14.7 | ± 5.9 | ± 3.8 |

Data indicate fibrinolytic activity (mm²) in the euglobulin fractions of plasma obtained from animals to which VIP, DS and UK had been intravenously administered. *P<0.05, **P<0.01 vs. corresponding zero time value (paired t-test).
to produce fibrinolysis. Since VIP did not show lysis in the plasminogen-rich bovine fibrinogen plate, unlike UK, VIP had neither a plasminogen activator nor plasmin activity. Enhanced fibrinolytic activity by VIP appeared very quickly after the intravenous injection in the present study. Perfusion studies using an isolated pig ear, demonstrated that vasoactive substances, such as acetylcholine, and dipyridamole, can enhance fibrinolytic activity by direct stimulation of the vascular wall (11). VIP, which relaxes the smooth muscle of blood vessels, may release plasminogen activators from endothelial cells associated with direct action on the vascular wall.

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References
1 Said, S.I. and Mutt, V.: Polypeptide with broad biological activity: isolation from small intestine. Science 169, 1217–1218 (1970)
2 Said, S.I. and Rosenberg, R.N.: Vasoactive intestinal polypeptide: abundant immunoreactivity in neural cell lines and normal nervous tissue. Science 192, 907–908 (1976)
3 Larsson, L.-I., Fahrenkrug, J., Scheffalitzky de Muckadell, O., Sundler, F., Hakason, R. and Rehfeld, J.F.: Localization of vasoactive intestinal polypeptide (VIP) to central and peripheral neurons. Proc. Natl. Acad. Sci. U.S.A. 73, 3197–3200 (1976)
4 Said, S.I.: Vasoactive intestinal polypeptide (VIP): current status. Peptides 5, 143–150 (1984)
5 Liu, Y.-X., Kasson, B.G., Dahl, K.D. and Hsueh, A.J.: Vasoactive intestinal peptide stimulates plasminogen activator activity by cultured rat granulosa cells and cumulus-oocyte complexes. Peptides 8, 29–33 (1987)
6 Rijken, D.C., Wijngaards, G. and Welbergen, J.: Relationship between tissue plasminogen activator and the activators in blood and vascular wall. Thromb. Res. 8, 815–830 (1980)
7 Gallimore, M.J., Tyler, H.M. and Shaw, J.T.B.: The measurement of fibrinolysis in the rat. Thromb. Diath. Haemorrh. 26, 295–310 (1971)
8 Van Der Graat, F., Keus, F.J.A., Vlooswijk, R.A.A. and Bouma, B.N.: The contact activation mechanism in human plasma: Activation induced by dextran sulfate. Blood 59, 1225–1233 (1982)
9 Tankersley, D.L. and Finlayson, J.S.: Kinetics of activation and autoactivation of human factor XII. Biochemistry 23, 273–279 (1984)
10 Niewiarowski, S. and Prou-Wartelle, O.: Role du facteur contact (facteur Hageman) dans la fibrinolyse. Thromb. Diath. Haemorrh. 3, 593–603 (1959)
11 Nakajima, K.: Pharmacological observations of plasminogen activator release caused by vasoactive agents in isolated perfused pig ears. Thromb. Res. 29, 163–174 (1983)