0014-4754/93/030242-03$1.50 + 0.20

Substance P-induced fibrinolysis in the forearm of healthy humans

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Received 23 October 1992; accepted 11 December 1992

Abstract. Physiological saline with or without substance P (50 ng/ml) was infused into the humeral artery in 6 healthy males. Indices of fibrinolytic activity (whole blood diluted lysis time, euglobulin lysis time, lysis areas in non-heated fibrin plates produced by plasma or euglobulin precipitate, plasminogen plasma levels, α_2-macroglobulin, C1-inhibitor, and α_2-antiplasmin) were evaluated in the homolateral antecubital vein before and after 5 min of substance P or saline infusion. After substance P the fibrinolytic activity increased, as can be seen from the shortening of lysis times ($p < 0.01$) and enlargement of the lysis areas ($p < 0.01$). A reduction of plasminogen plasma levels ($p < 0.01$), associated with a decrease in α_2-antiplasmin ($p < 0.01$), was also found. Alpha$_2$-macroglobulin and C1-inhibitor were instead unaltered by the peptide. The saline infusion, on the other hand, was unable to modify any of the examined indices. We concluded that exogenous substance P given intra-arterially increases fibrinolytic activity in locally-sampled venous blood through a mechanism which remains to be elucidated.
Key words. Substance P; fibrinolysis; plasminogen; α_2-antiplasmin; healthy humans.

Capsaicin, the active principle of hot peppers of the genus *Capsicum*, exhibits a broad bioactivity which includes fibrinolysis activation. *Capsicum* ingestion in fact appears to enhance the fibrinolytic activity in humans[1]. Furthermore, Thai people, who eat hot peppers daily, show a high fibrinolytic activity with reduced fibrinogen levels and hypocoagulability of the blood[2]. These phenomena could account for the rare occurrence of thromboembolic diseases among Thais.

The mechanism underlying the effect of *Capsicum* on fibrinolysis remains to be investigated. The ability of capsaicin to release substance P (SP) from sensory nerve endings has been well documented both in vivo and in vitro[3,4]. Moreover, a variety of responses to capsaicin in isolated organs may be ascribed to SP release from sensory nerves. In fact, exogenous SP produced a response in all these tissues which closely mimicked that of capsaicin[3]. In addition, many years ago we demonstrated using the thromboelastogram technique in humans that eleidosin, a peptide which acts on receptors for SP and related neurokinins[5], has fibrinolytic properties[6].

Direct evidence of SP action on the fibrinolytic system in man is still lacking. In the present investigation the SP effect on fibrinolysis was therefore evaluated in the antecubital vein after infusion of low doses of the peptide into the homolateral brachial artery.

Materials and methods

Subjects. The study was carried out on six healthy male volunteers chosen from the personnel of our Institute. After a complete explanation as to the aim and procedures of the trial, their informed consent was obtained. The investigation was fully approved by the Supervisory Committee of the Institute of Internal Medicine and Therapeutics IV of the University of Florence. Subjects' ages ranged between 26 and 37 years (average 33 years) and all were within the range of ideal body weight. They refrained from eating on the night before blood collection.

Blood sampling. At 09.00 h normal saline was infused for 5 min into the humeral artery of volunteers who had been lying down for at least 30 min, using an infusor pump (rate 1 ml/min). The saline was then immediately replaced by a 50 ng/ml SP solution for another 5 min. Blood samples were taken without venous stasis from the homolateral antecubital vein immediately prior to infusion and at the end of infusion of both saline and SP. Polypropylene syringes and 19 gauge needles were used. 9 ml of venous blood was immediately mixed with 1 ml of 3.8% sodium citrate, then centrifuged at 3000 rpm for 10 min to obtain plasma. Blood and plasma samples were kept in melting ice to be tested as soon as possible.

Fibrinolytic activity. The following assays were carried out to evaluate the fibrinolytic system:

1. The whole blood diluted lysis time (WBDLT) test was performed according to Fearnley et al.[7]. The lysis time reflects the fibrinolytic potentiality when activators and inhibitors are diluted.
2. The euglobulin lysis time (ELT) test performed according to Kluft and Brakman[8]. The plasma euglobulin fraction precipitated by acetic acid and centrifugation (3000 rpm for 15 min), containing plasminogen activa-

tors and some inhibitors, plasminogen, and fibrinogen, was redissolved and clotted with thrombin. The lysis time observed at 37 °C mainly depended on the amounts of plasminogen activators.

3. Lysis areas in non-heated fibrin plates were determined according to Nilsson et al.[9]. Those produced by plasma gave a measure of the fibrinolytic activity in the presence of both activators and inhibitors, and those produced by euglobulin precipitate (EP) gave an evaluation of the plasminogen activator activity after the removal of most of the plasminogen activator inhibitors.

4. Plasminogen plasma levels, α_2-macroglobulin and C1-inhibitor were evaluated with the immunoradial diffusion technique[10].

5. Alpha$_2$-antiplasmin was evaluated by the amidolytic method[11].

Drugs. Synthetic SP (25 µg lyophilisate per vial) was purchased from Clinalfa, Switzerland.

Statistical analysis. Statistical evaluation of the results was carried out with the Dunnett test. Values obtained before infusion were compared with those measured after saline and SP, respectively. Results are expressed as means ± SEM.

Results

Clinical effects. Unlike saline, SP infusion provoked oedema, which was limited to the treated forearm and lasted for about 10 min after infusion. No systemic clinical symptoms occurred.

Fibrinolytic activity. The saline infusion did not influence any of the tests of fibrinolytic activity which were studied: all the indices remained in the normal range (figs 1, 2; table).

After the end of SP infusion, shortening ($p < 0.01$) of WBLDT and ELT (fig. 1) and enlargement ($p < 0.01$) of lysis areas by plasma or EP (fig. 2) were observed.

A significant reduction in plasminogen levels ($p < 0.01$) associated with decrease of α_2-antiplasmin ($p < 0.01$) was also found (table).

Alpha$_2$-macroglobulin and C1-inhibitor were instead unaltered by SP (table).

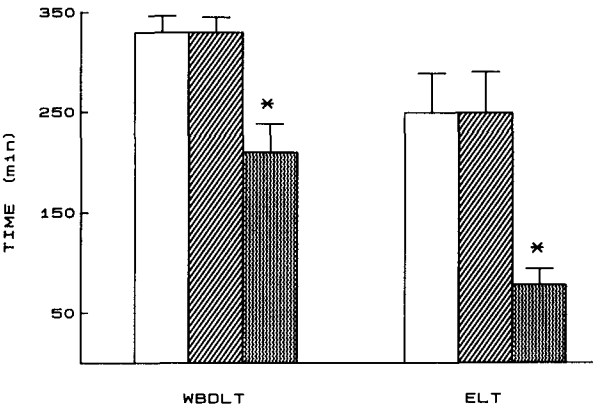

Figure 1. Whole blood diluted lysis time (WBDLT) and euglobulin lysis time (ELT) of the venous blood taken before (open bars) and after infusion of saline (dashed bars) or substance P (dotted bars) into the brachial artery of 6 healthy males. Values are means ± SEM. *$p < 0.01$ versus pre-infusion (Dunnett's test).

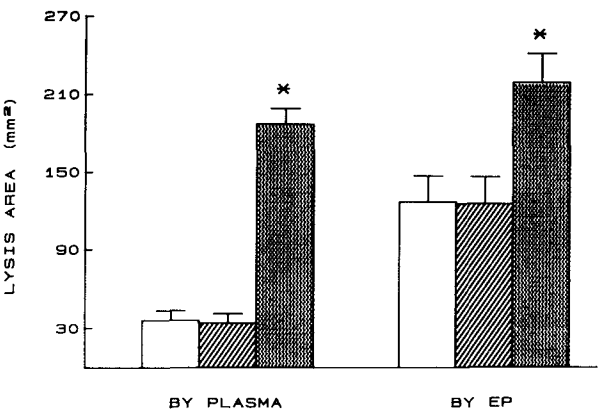

Figure 2. Lysis areas produced by plasma and by euglobulin precipitate (EP) obtained with the venous blood of 6 healthy males sampled before (open bars) and after arterial infusion of saline (dashed bars) and substance P (dotted bars). Data are means ± SEM. *$p < 0.01$ versus pre-infusion (Dunnett's test).

Discussion

The experimental procedure was designed to avoid any systemic effect in healthy volunteers treated with SP. Neutral endopeptidase, also called enkephalinase, plays a major role in SP inactivation. It has been shown to

Measurements of various components of the fibrinolytic system

Measurement	Reference value	Pre-infusion value	After saline	After SP
Plasminogen (mg%)	8 20	9.78 ± 0.16	9.82 ± 0.16 NS	9.31 ± 0.18*
α_2-antiplasmin (U/ml)	0.8–1.2	1.24 ± 0.08	1.25 ± 0.09 NS	0.85 ± 0.12*
α_2-macroglobulin (mg%)	150 420	153.80 ± 16.97	159.00 ± 19.90 NS	152.00 ± 11.28 NS
C1-inhibitor (mg%)	15–35	30.02 ± 1.89	30.05 ± 1.90 NS	31.75 ± 2.94 NS

Components were measured in venous blood of 6 healthy males before and 5 min after arterial infusion of saline or substance P (SP). Data are means ± SEM. *$p < 0.01$ and NS (not significant) versus pre-infusion value (Dunnett's test).

cleave efficiently various peptides, including SP[12]. Neutral endopeptidase inhibitors have also been demonstrated to potentiate both SP-induced effects[13] and the increase in SP-like immunoreactivity from sensory neurons[14]. Moreover, it is very likely that a release of the enzyme from the microvessels occurs, since neutral endopeptidase activity has been found to be much higher in venous than in arterial human plasma[15]. Therefore, arterial SP injection was considered a suitable route for exploiting the kinin-clearing capacity of the microcirculation. A final assurance of safety was the use of synthetic SP, which is much more rapidly destroyed in human plasma than endogenous SP[16,17].

The findings of the present study indicate that exogenous SP enhances plasma fibrinolytic activity. In fact, the lysis times, fibrin plate lysis areas, plasminogen and α_2-antiplasmin assays unequivocally point to fibrinolysis stimulation.

The highly significant shortening of ELT as well as the notable enlargement of the fibrin plate lysis area by EP suggest a major role of plasminogen activators in SP-induced fibrinolysis potentiation. Since a clear-cut reduction in plasminogen concentrations in human plasma occurred, a depletion of α_2-antiplasmin, the primary inhibitor of plasmin, was also expected.

However, neither of the natural circulating inhibitors, i.e. α_2-macroglobulin and C1-inhibitor, was altered by SP. Alpha$_2$-macroglobulin is a 'second defense line' inhibitor of many components of the fibrinolytic system such as plasmin, kallikrein, and the tissue-type plasminogen activator. It is likely that α_2-macroglobulin plays a role only after extensive activation of the fibrinolytic system, such as that which occurs during acute disseminated coagulation. C1-inhibitor inhibits plasma kallikrein and plasmin. By virtue of its inhibitory effect on components of the contact activation system and on plasmin it probably participates mainly in the inhibition of contact-dependent fibrinolysis.

Endothelial cells are the principal site of tissue-type plasminogen activator synthesis[18]. Moreover, SP has been found in the endothelial cells[19], where it induces vascular relaxation through stimulation of the neurokinin-1 receptor[20]. The hypothesis may therefore be advanced that the observed hyperfibrinolysis depends on release of tissue-type plasminogen activator from endothelial cells.

Our results are also in agreement with recent preliminary studies which indicate that intradermal SP increases fibrinolytic activity in the skin of healthy volunteers and patients suffering from necrotic vasculitis complicated with skin sores[21].

We thus conclude that arterially-administered SP activates the fibrinolytic system. Whether or not this phenomenon is linked to stimulation of the plasminogen activator remains to be clarified. Nevertheless, the results indicate that identification of new compounds capable of mobilizing endogenous SP or stimulating SP receptors might open up new prospects in fibrinolytic therapy. The beneficial effects obtained with the powerful vasodilating agent eledoisin[22], reported many years ago in some vascular diseases due to atherosclerosis[23], are very exciting in this connection, suggesting that the therapeutic action of SP receptor agonists derives from both vasodilatative and fibrinolytic properties.

Acknowledgments. This work was carried out as part of the Targeted Project 'Prevention and Control Disease Factors – F.A.T.M.A. (grant No. 91.00263.PF 41) of the National Research Council (C.N.R.), Rome, Italy. The authors wish to thank Mr Pasquale Imperiale for the technical help and illustrations.

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