

In vitro stimulation of plasminogen activator release from vein walls by adrenaline

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SUMMARY The effect of adrenaline on plasminogen activator release was studied in vitro in human vein biopsy specimens, in which the fibrinolytic activity was determined according to the fibrin slide technique. The tissue slides were covered with a thin fibrin film containing 10^{-9} and 10^{-7} M adrenaline and exposed for 30 to 60 minutes. In both concentrations highly significant ($p < 0.001$) enhancement of fibrinolytic activity was shown, and the enhancement of fibrinolysis was most pronounced during the first 30 minutes of exposure. Stimulation of fibrinolysis was maximal after exposure to the physiological concentration of 10^{-9} M, while no further increase was seen using the pharmacological concentration. These results show that adrenaline has a stimulant effect on tissue fibrinolysis in vitro, and this effect may account for the direct stimulation of fibrinolysis by adrenaline in vivo.

The fibrin slide technique originally described by Todd in 1958 is a widely used histochemical method for showing fibrinolytic activity in tissues.¹ By improving histochemical procedures and criteria for reading slides, this qualitative technique can be used as a semiquantitative method for assessing of plasminogen activator content in various tissues.² Using the modification by Pandolfi *et al*,³ it has been shown that impaired fibrinolytic activity in vein walls predisposes to thromboembolic complications.⁴⁻⁶ Furthermore, both stimulation and inhibition of vein wall fibrinolysis after treatment with steroids has been shown.⁷⁻⁹

In tissues and body fluids two immunologically and structurally different plasminogen activators have been recognised—that is, urokinase and tissue type plasminogen activator. According to immunohistochemical studies, however, the plasminogen activator in the vein wall has been characterised as a tissue type plasminogen activator, while no activity of urokinase could be shown.^{10,11} Tissue type plasminogen activator is also released into the blood stream after infusion of desamino-8-D-arginine vasopressin (DDAVP) and on stimulation with vasoactive drugs.¹²⁻¹⁵

In perfusion studies of isolated organs such as rat

hind leg and pig ear, vasoactive drugs such as bradykinin, acetylcholine, and adrenaline enhance plasma fibrinolysis by direct stimulation of vascular plasminogen activator release.¹³⁻¹⁷ The aim of this investigation was to study the in vitro effect of adrenaline on vein wall fibrinolysis by adding it to the fibrin film covering the tissue slices.

Material and methods

The present series included 20 healthy regularly menstruating women aged 34-51 years (median age 41 years) who were admitted to Huddinge University Hospital for determination of fibrinolytic activity in vein walls. After their informed consent data on age, menstrual cycle, smoking habits, and use of oral contraceptives (including duration and type) were registered before the sampling took place. Four women were cigarette smokers (< 10 cigarettes daily) and six were using combined oral contraceptives. The remaining 10 non-smokers who had never used oral contraceptives served as controls.

In each woman a superficial vein on the dorsal foot was prepared by gentle dissection under local anaesthesia of the surrounding connective tissue containing 2 ml 0.5% prilocain (Citanest, Astra). The vessel specimens, about 0.5 cm in length, were immediately frozen in liquid nitrogen and stored (-20°C) until analysed for fibrinolytic activity.

DETERMINATION OF THE PLASMINOGEN
ACTIVATOR ACTIVITY

Fibrinolytic activity in the vein walls was assayed according to the semiquantitative technique previously described by Pandolfi.³ Briefly, five thin (8 µm) cross sections of the vessel specimens were placed on a glass slide and covered with a thin plasminogen rich fibrin film with and without adrenaline. The fibrin film was obtained by mixing 0.06 ml human fibrinogen (1%, KabiVitrum) and 0.01 ml thrombin (Topostasin Roche, 20 National Institute of Health U/ml saline). After stabilisation at room temperature (21°C) for half an hour the fibrin slides were incubated in a moist chamber (37°C) for 0, 10, 20, and 30 minutes, and fixed in formalin. The slides were then stained with Giemsa solution and Harris's haematoxylin. Fibrinolytic activity was evident by zones of clearing in the fibrin film and estimated blindly by the same laboratory assistant according to a four graded scale (0 = no lysis; 1 = microscopic punctate areas of lysis; 2 = gross lytic areas; 3 = dissolution of most or all fibrin). The fibrinolytic activity in the vein walls was expressed in arbitrary units by summarising the scores of the four different incubation periods,^{2,3} the maximum score thus being 12 arbitrary units.

EXPOSURE TO ADRENALINE

Adrenaline (1 mg/ml, Astra) was diluted 1/2400 and 1/240 000 in saline, and 0.1 ml of these freshly prepared stock solutions was added to 2 ml fibrinogen. Thus the final concentration of adrenaline was 10^{-9} and 10^{-7} M, respectively, in the fibrin film covering the exposed cryostat sections.

STATISTICS

Non-parametric tests—that is, the Kruskal-Wallis one way analysis of variance and the sign test—were used for the statistical evaluation of the results.¹⁸

Results

Table 1 shows the mean fibrinolytic activity in human foot veins with and without exposure to adrenaline. In preparations with physiological concentrations of

Table 1 Fibrinolytic activity in vein biopsy specimens after *in vitro* exposure to adrenaline

	Concentration of adrenaline (mol/l) in fibrin film		
	0	10^{-9} M	10^{-7} M
No	20	20	20
Mean	4.8	5.8	5.5
SD	0.6	0.6	0.7
Analysis of variance		p < 0.001	

adrenaline (1 nmol/l) a highly significant ($p > 0.001$) increase in fibrinolytic activity was recorded. After addition of the pharmacological dose (100 nmol/l) the increase was still highly significant ($p > 0.001$), but no further stimulation of vein fibrinolysis was seen. Smokers and users of oral contraceptives did not differ from controls with respect to vein wall activity and fibrinolytic response to adrenaline.

Table 2 shows the data on the relation between incubation period and stimulating effect of adrenaline on vein wall fibrinolysis. During preincubation for 30 minutes at 21°C, a highly significant increase in fibrinolytic activity was seen in preparations exposed to the physiological concentration, and a significant increase was also seen in the pharmacological concentration. During subsequent incubation at 37°C for 10, 20, and 30 minutes, a further small but consistent increase of about 0.30 arbitrary units was recorded, irrespective of the adrenaline concentration used. Thus the increase in fibrinolytic activity recorded in vein biopsy specimens exposed to adrenaline mainly reflects the stimulating effect during the preincubation period.

The fibrin slides illustrate the typical findings in the present series (fig 1). In this woman no activity was seen in the control preparation, while distinct lytic areas were clearly visible after incubation at 21°C with exposure to adrenaline. After incubation at 37°C, however, no significant difference between the exposed and unexposed vein biopsy specimens could be seen.

Table 2 Increase of vein wall fibrinolysis after *in vitro* exposure to adrenaline during preincubation (30 minutes) and subsequent incubation at 37°C

Adrenaline concentration	Increase in fibrinolytic activity (arbitrary units)			Total Mean (%)
	After preincubation	During incubation	Total	
	Mean	Mean	Mean	
10^{-9} M	0.70*	0.30	1.0*	(20.8)
10^{-7} M	0.40†	0.35	0.75*	(14.8)

*p < 0.001; †p < 0.05.

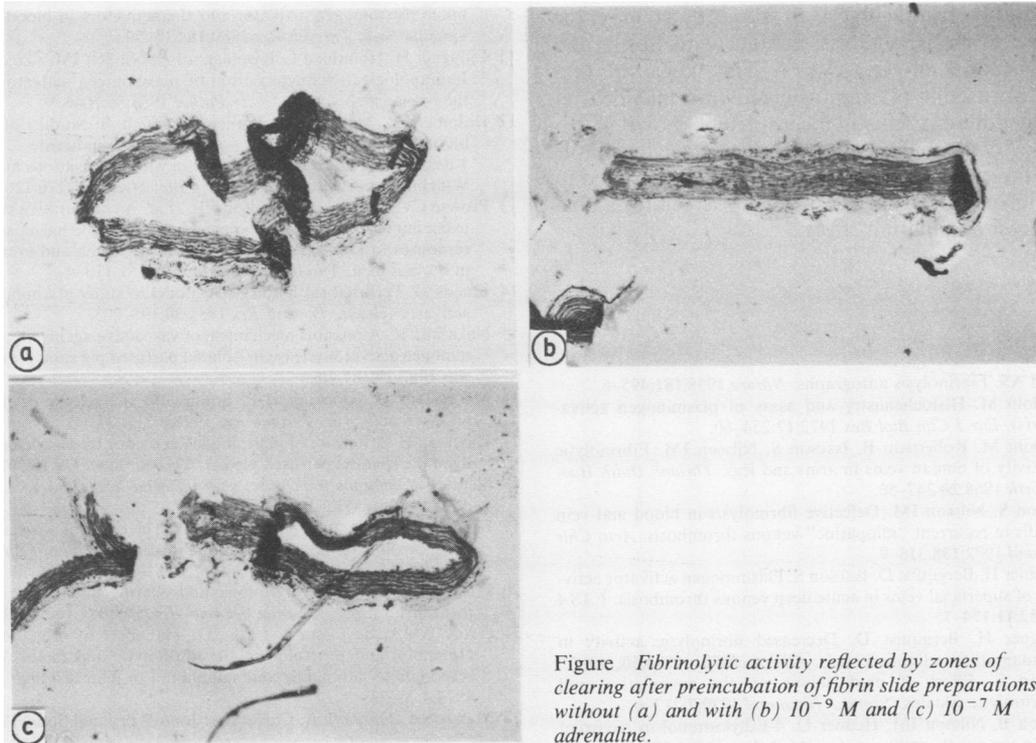


Figure Fibrinolytic activity reflected by zones of clearing after preincubation of fibrin slide preparations without (a) and with (b) 10^{-9} M and (c) 10^{-7} M adrenaline.

Discussion

The present study shows that the fibrinolytic activity in vein walls can be stimulated *in vitro* by adrenaline added to the fibrin film covering the tissue slices. This enhancing effect on fibrinolysis was already demonstrable in the concentration of 10^{-9} M, at which plasma concentration of adrenaline stimulates both β adrenergic (vasodilatation) and α adrenergic (vasoconstriction) receptors in man.¹⁹ Thus vein biopsies for clinical use should be taken during resting conditions in a relaxed patient, as the fibrinolytic activity in patients with vasoconstriction may be increased due to circulating adrenaline. The fibrinolytic response was maximal after exposure to the physiological concentration (10^{-9} M), although similar stimulation of the fibrinolytic activity was found using the pharmacological concentration of 10^{-7} M. This concentration is about 200 times lower than that commonly used in local anaesthetic preparations (4–5 μ g adrenaline/ml). Accordingly, preparations containing adrenaline or other vasoconstrictive drugs should be avoided if sampling for fibrinolytic studies, considering the strongly stimulating effect of adrenaline on veins exposed to considerably lower adrenaline concentrations.

In the vein biopsy specimens exposed to adrenaline

fibrinolysis was clearly stimulated during preincubation, while no significant effect was shown after incubation at 37°C. This may be explained by the fact that, normally, little or no fibrinolytic activity in dorsal foot veins is present after the preincubation period of 30 minutes at room temperature, whereas several distinct lytic areas already appear after the shortest incubation period (10 minutes). Thus the stimulating effect of adrenaline was more easily recognised in preparations with little or no activity than in those with more pronounced activity. This suggests that the release of plasminogen activators induced by adrenaline is relatively small compared with the release dependent on temperature during incubation. Therefore the usefulness of adrenaline as an agent to determine *in vitro* fibrinolytic release capacity may be limited compared with other substances known to have a high *in vivo* effect, such as DDAVP.²⁰

Our findings of initial stimulation of fibrinolysis in veins exposed *in vitro* indicate that adrenaline enhances fibrinolysis by direct stimulation of plasminogen activator release from endothelial cells. In man, however, administration of β adrenergic antagonists only partially inhibits the adrenaline induced enhancement of fibrinolysis.²¹ Furthermore, the fibrinolytic response to catecholamines and physiological stress such as exercise and smoking may vary

considerably from subject to subject, as may the response to stasis, acidosis, contact with fibrin, and administration of DDAVP.²² This suggests that release of vascular plasminogen activator may be triggered by different but still incompletely known mechanisms.^{14 15} Probably the outlined technically simple method using in vitro exposure may also be applied to other fibrinolytically active agents to screen for a possible direct stimulating effect.

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