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HUMAN PLASMINOGEN ACTIVATOR FROM HEART AND VASCULAR ENDOTHELIUM. N.A. Booth, B. Bennett. Department of Medicine, University of Aberdeen, Scotland.

Plasminogen activators play a central role in fibrinolysis. At present little is known about the interrelationships and molecular properties of these protein(s). There have been conflicting reports on the number of polypeptide chains present in the active molecules; plasminogen activators, purified from human uterus and human plasma after venous occlusion, are reported to consist of two chains, each of approximately 30,000 MW, linked by disulphide bonds. In contrast, the protein purified from human vascular endothelium consists of a single chain of 67,000 MW. Evidence for proteolytic activation of a single chain precursor to a two-chain form has been obtained by Wallen, in that the protein could be isolated in the single chain form only if inhibitors of proteolysis were present throughout purification. The two-chain form was found to possess significantly greater activity. By analogy with other plasma systems, generation of an active two-chain form from a single-chain precursor may serve a regulatory function, but the role of limited proteolysis of plasminogen activator remains to be elucidated.

Using modifications of reported methods, we have obtained improved purification of plasminogen activator from human heart and vascular endothelium by similar procedures. This has allowed detailed comparison of the enzyme from these two sources, in terms of chain structure, active site labelling and carbohydrate content. In particular, the effect of variation of conditions during purification on the chain structure of the isolated molecule has been studied.

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LOCAL VERSUS SYSTEMIC FIBRINOLYTIC THERAPY. V.A. Stowell, J.E. Mitchell, J.A. Caprini, J.P. Vagher, L. Zuckerman. Department of Surgery, Evanston Hospital, Evanston, Illinois, USA.

The purpose of this study was to examine fibrinolytic activation produced by streptokinase-plasmin complex in a dog with an artificially-induced thrombus. A labelled thrombus was produced by clotting a Wessler stasis segment in the external jugular vein with thrombin (100 u) and I-125 Fibrinogen (100 μC) and occlusion maintained for 40 minutes. Streptokinase-plasmin complex (SKp) or saline was injected as a systemic bolus (femoral vein) or locally (at the jugular site) 60 minutes after the thrombus was formed. Clot dissolution was monitored by appearance of I-125 in the circulation. Platelet count, hematocrit, partial thromboplastin time, fibrinogen, fibrin split products, thrombelastography, and Lee-White clotting times were performed before the surgical procedure and at 30 minute intervals. A systemic fibrinolytic state was achieved in both local and systemic experiments. However, I-125 appearance and gross examination after the experiments showed that no appreciable clot dissolution had occurred when SKp was injected systemically in contrast to virtually complete lysis with local SKp injection. This total dissolution was apparent 40 minutes post injection. It was concluded that I-125 appearance is a sensitive monitoring method for detecting the degree of lysis, and local administration of the fibrinolytic agent seems to be much more effective than systemic SKp injection.

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THE FIBRINOLYTIC RESPONSE TO STANOZOLOL IN NORMAL SUBJECTS. M. Greaves and F.E. Preston, University Department of Haematology, Royal Hallamshire Hospital, Sheffield, U.K.

It has been recognized for a number of years that fibrinolytic activity in patients with various forms of vascular disease may be enhanced by stanozolol (17-β-hydroxy-17α-methyl adrostano {3.2,- c } pyrazole). However, little is known of its effect in normal healthy individuals. Also it is not known how quickly the drug exerts its effects. Two groups of eight normal healthy adults were studied. Seven subjects were included in both groups. Group A received stanozolol 5mgs b.d. Group B received 5mgs daily. Before receiving the drug, two base line studies were performed on blood samples from each individual. Thereafter, blood samples were taken at 2, 7, 10, 17, 24, 31 and 38 days after commencement of treatment. The following investigations were performed - haemoglobin, white cell count, platelet count, euglobulin clot lysis time (ECLT), fibrinogen (Fg), plasminogen (Pg), α2 macroglobulin (α2M), fibrin degradation products, serum albumin, liver enzymes. In Group A statistically significant increases in plasminogen activator activity, measured by the ECLT, and Pg were observed 2 days ($P<0.05$) and 10 days ($P<0.05$) respectively after administration of stanozolol; these remained elevated throughout the study. Also, significant reductions of plasma Fg ($P<0.01$) and α_2M ($P<0.05$) occurred within 7 days of commencement of the drug and these also persisted throughout the six week period of the trial. Similar statistically-significant changes were also observed in Group B subjects in ECLT, Pg, α_2M, although the induced changes were less predictable than those observed in Group A. These results indicate that stanozolol exerts significant and persistent enhancement of fibrinolysis in normal individuals.

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KINETIC CHARACTERIZATION OF THE FAST ACTING PLASMIN INHIBITOR FROM HUMAN PLATELETS. M. Sandbjerg Hansen and Lars Chr. Petersen. Department of Clinical Chemistry, Hvidovre University Hospital, Copenhagen, Denmark.

An inhibitor of the plasma proteinase plasmin (EC 3.4.21.7) was partially purified from washed and lysed human blood platelets. The presence of the known plasma proteinase inhibitors in the preparations investigated was excluded by crossed immunoelectrophoresis and electroimmuno assay. The kinetics of the reaction between the inhibitor preparation and plasmin has been investigated using the synthetic chromogenic substrate S-2251 (D-Val-Leu-Lys-pNA).

Residual plasmin activity was measured varying the amounts of inhibitor at various fixed concentrations of substrate (S-2251). The inhibitor is of the noncompetitive type as indicated by a Dixon-plot. The reaction between inhibitor and plasmin is fast with an estimated dissociation constant of approximately. 0.1. nM. The reaction is reversible as evidenced by the following findings: 1. inhibition in the presence of high concentrations of substrate is a fast reaction, but total inhibition of plasmin is not obtained, 2. inactivation of plasmin as a function of inhibitor concentration does not follow a simple titration curve, 3. addition of tranexamic acid increases the apparent K_i, 4. the complex beetween the inhibitor and plasmin is demonstrable in crossed immunoelectrophoresis but not in SDS-polyacrylamide gel electrophoresis. The results suggest that the platelet plasmin inhibitor is a reversible inhibitor with a high affinity for plasmin.