REGULATION OF FIBRINOLYSIS THROUGH ACTIVATION AND INHIBITION OF ACTIVATION OF A PLASMINOGEN PROACTIVATOR (PREUKINASE). M.B. Bernik and E.P. Oller, Northwestern University Medical School, Chicago, Illinois U.S.A.

Purified preparations of a plasminogen proactivator, preukinase (preUK), the enzyme plasmin and thrombin and native inhibitors of these enzymes (C1'-esterase inhibitor and antithrombin III) were used to study factors and events which may participate in the regulation of fibrinolysis by facilitating or hindering conversion of preUK to UK. PreUK, expressed in CTA units of generated UK, was purified from supernates of kidney cultures by chromatography on Sephadex G-100 columns to specific activity of 20,000 CTA U/mg protein and was studied by immunodiffusion and immunoelectrophoresis with antisera to highly purified urinary and tissue culture UK. In assays on fibrin plates, generation of UK activity (3-4 CTA U/ml) from pre UK occurred with traces of plasmin (0.01 CTA U/ml) or trypsin (10 BAEE U/ml) but not with thrombin (0.01-100 NIH U/ml). Inhibition of UK generation occurred in two ways: 1) by inhibition of the activating enzymes, and 2) by inactivation of pre UK itself. The former was demonstrated with C1'-esterase inhibitor which inhibited the fibrinolytic as well as preUK-activating properties of plasmin and trypsin, and the latter with thrombin which inactivated preUK progressively in a dose-response fashion without being itself consumed. Thus, 3 CTA U/ml preUK were inactivated in 20 min or less at 37°C by 2.5 NIH U/ml thrombin and in more than 3 hr by 0.01 NIH U/ml. Thrombin-induced preUK inactivation was inhibited by antithrombin III indicating that while thrombin acted as an inhibitor of fibrinolysis by inactivating preUK, antithrombin III facilitated fibrinolysis indirectly by protecting preUK from thrombin.

THE ACTIVATION OF PLASMINOGEN BY HAGEMAN FACTOR AND HAGEMAN FACTOR FRAGMENTS. G. Goldsmith, H. Saito and O.D. Ratnoff. Case Western Reserve University School of Medicine, Cleveland, Ohio.

The mechanism of action of Hageman factor (HF, Factor XII) in surface-mediated conversion of plasminogen to plasmin was investigated, assaying plasmin with H-D-Valyl-L-leucyl-L-lysine p-nitroanilide-2HCL. Unexpectedly, amidolytic activity for this substrate evolved in the presence of kaolin in mixtures of purified preparations of plasminogen and HF, both devoid of detectable prekallikrein and high MW kininogen. The same result was obtained when plasminogen preparations were incubated in the absence of kaolin with HF-fragments prepared by digestion of HF with insoluble trypsin or with gel-filtered HF fragments (approximate MW 30,000). High MW kininogen did not enhance amidolysis in mixtures of plasminogen and HF with kaolin or HF fragments, but both amidolysis and fibrinolysis were enhanced by the fraction of normal plasma not absorbed at low ionic strength to QAE-Sephadex gels at pH 7.5. The analogous fraction of Fletcher trait (prekallikrein deficient) plasma, did not enhance amidolysis or fibrinolysis. Kinetic study of the HF-kaolin-plasminogen interaction indicated a direct enzymatic role for the HF moiety acting on plasminogen as a substrate.

These data suggest that, contrary to earlier observations, HF or HF-fragments may play a direct role in the activation of plasminogen.

HUMAN PREKALLIKREIN (PLASMINOGEN PROACTIVATOR): PURIFICATION, CHARACTERIZATION AND ACTIVATION BY ACTIVATED FACTOR XII. Bonno N. Bouma and John H. Griffin. Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, California, U.S.A.

In order to resolve conflicting reports about the possible identity of prekallikrein and Factor XII-dependent plasminogen proactivator (FXII-PPA), the γ -globulin fractions of prekallikrein-deficient (Fletcher trait) and of normal plasma were assayed for FXII-PPA. Based on both fibrin plate and clot lysis tests, FXII-PPA in the γ -globulin fractions of prekallikrein-deficient plasmas from 2 unrelated patients was undetectable, i.e. <1% of the FXII-PPA in the normal γ -globulin fraction. However, PPA independent of FXII was detected in both the Fletcher and the normal γ -globulin fractions at 4% of the FXII-PPA present in the normal γ -globulin fraction.

Human plasma prekallikrein was purified 2,000-fold (specific clotting activity 22 units/mg) and was greater than 95% homogeneous on SDS-gels. FXII-PPA was always copurified with prekallikrein and was totally separated from Factor XI. No Factor XII-dependent or Factor XII-independent plasminogen activator activity was detected in purified Factor XI preparations at 40 units/ml. Purified prekallikrein in its precursor form gave 2 protein bands on SDS-gels at 82,000 and 78,000 MW. Upon reduction, a single 85,000 MW band was observed. Kallikrein and plasminogen activator activity were generated upon incubation with purified human Factor XII_a (28,000 MW form). Analysis of this reaction mixture on SDS-gels without reduction showed 2 bands with apparently identical MW's as the precursor protein bands, whereas reduction showed cleavage of both protein bands.

These results suggest that prekallikrein is identical to FXII-PPA in normal human plasma and that activation of this zymogen by Factor XII_a involves limited proteolytic cleavage.