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AUTOACTIVATION OF HUMAN PLASMA PREKALLIKREIN. G. Tans (1), J. Rosing (1), M. Berrettini (2), B. Lämmle (2) and J.H. Griffin (2). University of Limburg, Maastricht, The Netherlands (1) and Scripps Clinic and Research Foundation, La Jolla, CA., USA (2).

Incubation of purified human plasma prekallikrein with sulfatides or dextran sulfate resulted in spontaneous activation of prekallikrein as judged by the appearance of amidolytic activity towards the chromogenic substrate H-D-pro-phe-arg-p-nitroanilide (S 2302). The time course of generation of amidolytic activity was sigmoidal with an apparent lag phase followed by a rapid activation until finally a plateau was reached. Soybean trypsin inhibitor completely blocked prekallikrein activation whereas corn, limabean and ovomucoid trypsin inhibitor did not. The K_i of the reversible inhibitor, benzamidine, for autoactivation (240 uM) was identical to the K_i of benzamidine for kallikrein. Thus, spontaneous prekallikrein activation and kallikrein showed the same specificity for a number of serine protease inhibitors, indicating that prekallikrein is activated by its own enzymatically active form, kallikrein. Immunoblotting analysis showed that, concomitant with the appearance of amidolytic activity, prekallikrein was cleaved. However, prekallikrein was not quantitatively converted into two-chain kallikrein since other polypeptide products were visible on the gels. This accounts for the observation that in amidolytic assays not all prekallikrein present in the reaction mixture was measured as active kallikrein. Kinetic analysis showed that prekallikrein activation can be described by a second-order reaction mechanism in which prekallikrein is activated kallikrein. The apparent second order rate constant was 27000 M-1s-1 (pH 7.2, 50 uM sulfatides, ionic strength I=0.06, at 37 °C). Autocatalytic prekallikrein activation was strongly dependent on the ionic strength, since there was a considerable decrease in the rate of the reaction at high salt concentrations. Our data support a prekallikrein autoactivation mechanism in which surface-bound kallikrein activates surface-bound prekallikrein. The rate constant of autoactivation is considerably lower than the rate constants reported for Factor XIIa dependent prekallikrein formation. Autocatalytic prekallikrein activation may, however, contribute to kallikrein formation during the initiating phase of contact activation.

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INTERACTIONS OF ACTIVATED HAGEMAN FACTOR (Factor XIIa, HFa) WITH NORMAL $C\bar{1}$-INHIBITOR. V.H. Donaldson (1), B.H. Mitchell (1), and O.D. Ratnoff (2). University of Cincinnati, Children's Hospital Research Foundation, Cincinnati, OH U.S.A. (1); and University Hospitals of Cleveland, Western Reserve University, Cleveland, OH U.S.A. (2).

Purified Hageman factor activated with a mixture of brain sulfatides and bovine serum albumin (BSA) developed clot-promoting activity which was inhibited by purified normal human $C\bar{1}$-inhibitor, but during extended incubation there was an apparent loss of inhibition in these mixtures. When a preparation of Hageman factor fragments (HFf), which had a much lower specific Hageman factor coagulant activity, was incubated with BSA-sulfatides, its coagulant activity was also enhanced and could be inhibited by normal $C\bar{1}$-inhibitor. When the HFa having a molecular weight of approximately 75,000 was mixed with the HFf preparation before $C\bar{1}$-inhibitor was added, there was a progressive shortening of the Hageman factor specific clotting times with increasing amounts of HFf in the mixtures, despite the addition of $C\bar{1}$-inhibitor. Moreover, as the amounts of HFf in the mixtures were increased, a high molecular weight complex of about 190,000 containing HFa and $C\bar{1}$-inhibitor could no longer be identified in SDS-gel electrophoresis but a low molecular weight band (127,000) appeared, which probably contained HFf of about 22,000 molecular weight and $C\bar{1}$-inhibitor. Therefore, HFf appeared to compete effectively with the HFa (75,000) for $C\bar{1}$-inhibitor in these mixtures of purified reagents. It is also possible that HFf may have interacted with the HFa or the $C\bar{1}$-inhibitor changing the steric configuration of either one so that an interaction between HFa and $C\bar{1}$-inhibitor did not occur.

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FORMATION OF Cl_s-Cl INHIBITOR AND KALLIKREIN-Cl INHIBITOR COMPLEXES DURING CARDIOPULMONARY BYPASS. Yanina T. Wachtfogel (1), Peter C. Harpel,(2) L. Henry Edmunds, Jr.,(3) and Robert W. Colman (1). Temple University School of Medicine,Thrombosis Research Center, Philadelphia, PA, USA (1), Cornell University Medical Center, Thrombosis Research Center, New York, NY, USA (2), and University of Pennsylvania, Department of Surgery, Philadelphia, PA, USA (3)

Cardiopulmonary bypass prolongs bleeding time and increases postoperative blood loss. Contact of blood with synthetic surfaces during extracorporeal circulation leads to major qualitative and quantitative alterations in both platelets and neutrophils. Activation of platelets results in thrombocytopenia, decreased sensitivity of platelets to aggregating agents, decreased $alpha_2$-adrenergic and fibrinogen receptors, secretion of thromboxane B_2, and depletion of alpha-granule protein contents. Neutrophils,under similar conditions, have also been shown to release their specific granule protein, lactoferrin, and their azurophilic granule enzyme, elastase.We now investigate whether the classical complement, contact, or fibrinolytic pathways have been activated as potential sources of neutrophil agonists. Employing enzyme-linked immunosorbent "sandwich" assays specific for Cl_s-Cl inhibitor and kallikrein-Cl inhibitor complexes respectively, we found that plasma levels of both of these formed complexes increased 2-fold after clinical cardiopulmonary bypass was completed and reverted to baseline within 24 hours post-operatively. Since these complexes are cleared in vivo, we investigated their plasma levels during in vitro simulated extracorporeal circulation. Over a period of 2 hours, Cl_s-Cl inhibitor complexes rose from a baseline of 2 + 1nM to 21 + 2 nM and kallikrein-Cl inhibitor complexes rose from 2 + 1 nM to 25 + 5 nM. However, there was no evidence of either in vivo or in vitro plasmin-$alpha_2$ plasmin inhibitor complex formation. These results indicate that activation of the classical pathway of complement and the contact system in plasma may be associated with neutrophil activation seen during clinical cardiopulmonary bypass.

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MONOCLONAL ANTIBODY 13G11 RECOGNIZES PREKALLIKREIN, KALLIKREIN AND COMPLEXES OF KALLIKREIN WITH $C\bar{1}$-INHIBITOR AND α_2-MACROGLOBULIN. D. Veloso(1), M. Shapira(2), F. Kueppers(1) and R.W. Colman(1). Thromb. Res. Ctr., and Dept. Med.,Temple Univ. Sch. Med., Philadelphia PA(1) and Vanderbilt Univ., Nashville TN(2), U.S.A.

Abnormal prekallikrein (PK) levels in plasma can be due to decreased biosynthesis or increased activation either by surface-activated factor XIIa (e.g., in septicemia) or by other proteases (e.g., in pancreatites). To study the products of activation of PK in plasma, intact normal plasma and plasma exposed to either activating surfaces or factor XII fragment, was immunoblotted from SDS-gels. MAb 13G11 which recognizes purified PK, kallikrein (KAL) and the complexes of KAL with Cl-inhibitor (Cl-Inh) and α_2-macroglobulin (α_2M) formed from purified proteins detected a doublet (88- and 85-kDa) which comigrated with PK and KAL but was not visible in PK-deficient plasma. Transfer of either PK in normal plasma (25-125 ng) or KAL (50-300 ng) added to PK-deficient plasma was proportional to the amount of protein applied to the SDS-gels. Activation of plasma decreased the intensity of the PK bands with the formation of new bands with molecular weights similar to those of KAL-Cl-Inh and KAL-α_2M. Identity was confirmed by MAb 4C3 (reacts with KAL-Cl-In, not with KAL) and a polyclonal antibody to α_2M. Increase of incubation temperature from 24° to 37° increased KAL-Cl-Inh and decreased KAL-α_2M. Addition of an excess of α_2M before surface activation caused an increase of KAL-α_2M complex and a decrease of KAL-Cl-Inh. Addition of an excess of Cl-Inh increased KAL-Cl-Inh and decreased KAL-α_2M. In addition, activation of Cl-Inh-deficient plasma showed lower KAL-Cl-Inh and higher KAL-α_2M than those when normal plasma was activated. When the deficient plasma was treated with CH_3NH_2 to inactivate α_2M, an increase at KAL position was observed since no inhibitors were active. These studies indicate that 13G11 will be useful to detect changes in the distribution of PK, KAL, KAL-Cl-Inh and KAL-α_2M associated with abnormal activation of PK and/or abnormal availability of inhibitors in disease.