0728

COAGULATION FACTORS ARE ACTIVATORS OF HUMAN PLASMA "PRORENIN". D.H. Osmond, S.R. Tatemichi, E.A. Wilczynski, and A.D. Purdon. Department of Physiology, University of Toronto, Toronto, Canada.

We have demonstrated that human plasma "prorenin", an inactive precursor of the blood pressure regulating enzyme renin, can be activated by cold, e.g. -4 to +4°C for 1-30 days (Can. J. Physiol. Pharmacol. 51:705, 1973). Several workers have reported cold activation of the coagulation system. Suspecting a link between these two cold-activated enzyme systems, we established that in factor XII deficient plasma, the rate of cold activation of prorenin is halved (Lancet i, 1313, 1978). Trypsinization of plasma can mimic within 1 minute the effect of prolonged cold (Circ. Res. Suppl. 1, 41:171, 1977), and can overcome specific coagulation factor deficiencies in varying degrees. FXII, VII, V, and especially FX deficient plasmas, all have subnormal basal active renin levels, implying an impaired state of prorenin conversion in vivo. FXII deficient plasma activates least by cold, suggesting special importance of FXII for operation of cold activation. All the plasmas activate better with 0.5 mg trypsin/ml plasma than with cold, except FX, suggesting that it especially mediates tryptic activation. Increasing the trypsin concentration corrects for factor deficiencies in varying degrees, implying some non-specificity and interchangeability of factor requirements for prorenin activation. Our data point to a hierarchy of factor importance, and to a "cascade" of prorenin activation, by which plasma renin content can be rapidly increased. Thus, plasma renin activity is a function of renal release of renin, plus renin formation from renal (and possibly extrarenal) prorenin by an activation process involving the coagulation system.

0729

ACTIVATION OF FACTOR XII BY DEXTRAN SULFATE: A CONVENIENT ASSAY FOR FACTOR XII. D.L. Tankersley, B.M. Alving, and J.S. Finlayson. Bureau of Biologics, Bethesda, MD, and Walter Reed Army Institute for Research, Washington, DC.

The rapid reciprocal activation of Factor XII and prekallikrein by negatively charged surfaces requires the presence of high M_r kininogen. Our studies have demonstrated that high M_r kininogen is not required for the activation of Factor XII by the soluble activator, dextran sulfate (M_r 500,000). Incubation of normal, prekallikrein deficient, Factor XI deficient, or high M_r kininogen deficient plasma (1:1000 dilution) with prekallikrein in the presence of dextran sulfate (5 μg/ml) resulted in the formation of kallikrein (~50% activation in three minutes) as determined by hydrolysis of D-Pro-Phe-Arg-p-nitroanilide. No activation (<0.5%) occurred in the absence of dextran sulfate, or with Factor XII deficient plasma. When mixtures of Factor XII deficient and normal plasma were assessed for the ability to generate kallikrein by this method, the amount of kallikrein produced in three minutes was directly proportional to the Factor XII content of the mixture. Purified Factor XII (20 ng/ml final concentration) similarly activated prekallikrein in the presence of dextran sulfate, but not in its absence. Studies of the time-dependence of the activation process demonstrated a short lag period followed by an abrupt increase in kallikrein concentration, suggesting that there is rapid, complete activation of Factor XII and subsequent, progressive conversion of prekallikrein to kallikrein by the Factor XIIa.

0730

DIRECT ASSAY FOR FACTOR XII IN PLASMA. T. Zuffi and R. Jordan. Cutter Laboratories, Incorporated, Berkeley, California.

A chromogenic substrate assay for FXII in plasma samples has been developed. The method involves 0°C activation of the contact factors with dextran sulfate, and a direct selective measurement of the FXIIa activity generated by hydrolysis of the synthetic substrate S2302 in the presence of the kallikrein inhibitor, soybean trypsin inhibitor. Using these procedures, the kinetics of both FXII and prekallikrein activation could be studied and it was found that the relative rates of activation of these two zymogens did not necessarily coincide.

When this dextran sulfate treatment of FXII or HMWK-deficient plasmas was tried, insignificant kallikrein or FXIIa activity was generated, demonstrating that contact activation was not occurring. These studies have shown that a dextran sulfate activation can be used as an assay procedure for FXII in plasma and can distinguish normal from certain deficient plasmas.

0731

STUDIES RELATED TO TOBACCO GLYCOPROTEIN: A CLAIMED ACTIVATOR OF COAGULATION, FIBRINOLYSIS, COMPLEMENT, KININ, AND A CLAIMED ALLERGEN. R.L.Bick, R.L.Stedman, P.L.Kronick, E. Hillman, and J. Fareed. San Joaquin Hematology Oncology Medical Group, California Coagulation Laboratories, Bakersfield, California, UCLA, Los Angeles, California, Franklin Institute Research Center, Philadelphia, Penn., and Loyola University, Chicago, Illinois.

Brown pigments from cured tobacco leaf, cigarette smoke condensates and saline extracts of smoke have been referred to as tobacco glycoprotein (TGP) and have been reported to produce allergic skin reactions, induce the formation of IgE antibodies, and to activate clotting factor XII and subsequently activate the fibrinolytic and complement system. These sequential pathological events have been claimed to represent the pathophysiological link between cigarette smoking and cardiopulmonary disease which is observed statistically. To more carefully investigate these claims TGP was extracted by the previously published method of Becker et. al. (Proc. Natl. Acad. Sci. 73:1712-1716, 1976) and the resultant material subjected to in vitro assays related to the claimed biological activities of TGP. It was found that TGP cannot be isolated following the above procedure; the final product obtained by the isolation procedure contains a contaminant introduced by PAGE separation. The contaminant (CMT) appears to be a linear polyacrylate. When this isolated material was subjected to clotting system assays it was found to complex with factor XII, depleting about 15% of factor XII from plasma and,thus, giving the superficial appearance of activating factor XII however, no such activation occurred as noted by non-shortening of the non-activated PTT system, and coagulation factor assays. CMT was found to have no activity on the complement, fibrinolytic, or kinin system. Acrylates are known allergens and thrombus-inducing agents, and these studies suggest that CMT, instead of TGP, has been isolated from cigarette derivitives by the above reported method and CMT rather than TGP has many of the biological activities previously, and perhaps mistakenly, ascribed to TGP.