0805

08:45 h

NATIVE FACTOR XII (FX11) REQUIRES PROTEOLYTIC CLEAVAGE FOR ACTIVATION. M. Silverberg & A.P. Kaplan. Health Science Center SUNY, Stony Brook, New York.

It has been proposed that FX11 zymogen can cleave prekallikrein without prior conversion into one of its proteolytically cleaved forms FX11a or FX11f. We treated FX11 with pro-phe-arg-chloromethyl ketone (PPACMK) to remove all measurable amidolytic activity due to traces of FX11a or FX11f. Prekallikrein (PK) and IgG were similarly treated to remove all traces of kallikrein. FX11 was diluted to 0.016 µg/ml, using 3.8mg/ml IgG as the diluent, and mixed 1:1 with 215µg/ml PK. The rate of generation of kallikrein activity was neglible to begin with but increased with time to approach the rate of activation obtained with FX11a or FX11f at equivalent concentrations. When 10µg/ml dextran sulphate was present the rate of PK activation was maximal from the beginning. This experiment was repeated in the presence of a specific inhibitor of activated FX11 derived from corn; 0.1mg/ml inhibitor prevented the activation of PK. This indicates that the PK activator is the same species that reacts with the inhibitor, namely FX11a or FX11f. In another experiment, 1.67µg/ml of PPACMK-treated FX11 and 2.6 µg/ml PPACMK-treated PK were mixed in glass cuvettes containing 500µM chromogenic substrates specific for either FX11a or kallikrein. The resulting curves of absorbance vs time showed that both FX11 and PK became activated in accelerating reactions after short lag phases; corn inhibitor blocked this reaction too. Thus the rapid activation and the lack of a lag phase seen in the experiment with dextran sulphate was not due to a substrate-induced formation of an active site in FX11 zymogen but was a result of the very rapid activation of surface bound FX11 by traces of kallikrein. The kallikrein may have been generated by traces of FX11a which are always present even after treatment of FX11 with inhibitors. This amount of FX11a may also be present in vivo and provides a potential mechanism for the initiation of contact activation.

0806

09:00 h

INFLUENCE OF OPERATIVE TRAUMA ON F XII AND INHIBITOR OF PLASMINOGEN ACTIVATOR. U. Hedner and D. Bergqvist. Department of Coagulation Disorders and Department of Surgery, Allmänna Sjukhuset, University of Lund, Malmö, Sweden

A parallel decrease of factor XII and an inhibitor of plasminogen activator (PA inhibitor) earlier described by Hedner (1973, 1980) was observed in a small series of patients postoperatively (Hedner 1978). In order to further investigate this, 52 patients (29 males and 23 females) were studied in connection with vascular surgery. The following parameters were analysed: factor XII (clotting and imm.chem.assays), PA inhibitor, α_2-antiplasmin (α_2AP), α_2-macroglobulin (α_2M) and C$\bar{1}$-inactivator (C$\bar{1}$-INA)(rocket technique) and factor IX (clotting assay). A significant parallel decrease to about 50 % of factor XII measured with both methods reaching its minimum on day 4-5 postop as well as of the PA inhib was seen. This decrease was found in all patient groups except those operated upon because of varicose veins. These latter patients all left the hospital already on the 2nd or 3rd postop day. No concomitant decrease of α_2AP, α_2M, C$\bar{1}$-INA was seen. The observed lowering of f XII and PA inhib on day 4-5 did not depend on changes in haematocrit associated with the operation.

The findings of a parallel decrease of f XII and the PA inhib shown to inhibit f XIIa (Hedner and Martinsson 1978) may indicate that an activation of f XII followed by consumption of this factor as well as of the inhibitor to f XIIa occurs during or after surgery. Whether the f XII dependent fibrinolysis is of importance in the defence against deep venous thrombosis is still debatable. It is, however, striking that low levels of f XII occurs postop at a time when most proteins show an increased synthesis rate because of stimulation by the acute phase reaction. Furthermore inhibitors against the activation phase is known to be associated with thrombotic disease (Hedner and Nilsson 1976).

0807

09:15 h

EFFECT OF TWO SOLUBLE "ACTIVATORS" ON THE ACTIVITY OF FACTOR XII AND FACTOR XIIa (HAGEMAN FACTOR) Judith S. Greengard and John H. Griffin, Research Institute of Scripps Clinic, La Jolla, California 92037 USA

Certain activators of the contact system of coagulation have been reported to induce full activity in the single-chain form (80,000 MW) of Factor XII (FXII). The effects of soluble ellagic acid (EA) and dextran sulfate (DXS) (~500,000 MW) on purified components of the contact system were studied. Soluble EA (<40 µM) was found to exert a dose-dependent procoagulant effect on purified FXII added to FXII-deficient plasma although the maximal activity observed was much less than that elicited by kaolin in the same mixtures. 5 µM soluble EA increased the amidolytic activity of FXII against H-D-Pro-Phe-Arg-pNA from 2.5 to 5.0 mol substrate/mol enzyme/min. Purified ß-FXIIa (28,000 MW) or FXII that had been preincubated with trypsin hydrolyzed 900 mol substrate/mol enzyme/min in the presence or absence of soluble EA. Thus, soluble EA induces minimal (≤0.3%) amidolytic activity in FXII. We also examined the generation of FXIa in the presence of 5 µM soluble EA, FXI, high MW kininogen, and FXII or α-FXIIa (two-chain form, 80,000 MW). The rate of cleavage of ^{125}I-FXI in the presence of 10 µg/ml DXS, FXI, high MW kininogen, and FXII or α-FXIIa was also measured. In the presence of either DXS or soluble EA under the concentrations and conditions employed, α-FXIIa exhibited an initial rate of FXI activation that was 20 times higher than that achieved by single-chain FXII. In addition, the presence of both high MW kininogen and either EA or DXS enhanced the activity of α-FXIIa 20 fold in the activation of FXI. The results show that single chain FXII in the presence of either DXS or soluble EA expresses less than 5% of the enzymatic activities of its proteolytically derived forms whereas either "activator" greatly enhances the action of α-FXIIa on FXI in the presence of high MW kininogen.

0808

09:45 h

INHIBITION OF CONTACT ACTIVATION BY PLATELET FACTOR 4. K. Weerasinghe, M.F. Scully, and V.V. Kakkar. Thrombosis Research Unit, King's College Hospital Medical School, London, England.

The contact activation of FXII by dextran sulphate (DS, MW 500,000) has been shown to be inhibited by platelet factor 4 (PF4). Plasma was prepared from blood taken with EDTA, theophylline and PGE_1. Contact activation was initiated by the addition of an equal volume of DS (10µg/ml) and incubating at 0° (8 min) or 37° (2 min). The kallikrein generated was measured using S2302. Addition of purified PF4 to plasma caused a concentration dependent inhibition with complete inhibition at greater than 2.0µg/PF4/ml plasma (molar ratio of 20:1). PF4 was also observed in a concentration dependent manner to inhibit clotting of plasma as initiated by dextran sulphate.

Repeated freezing of human PRP (Av. platelet count=3.55x 10^8/ml) released PF4 (as measured by radioimmunoassay) and caused inhibition of contact activation. By dilution with PPP, the degree of inhibition was found to be related to the concentration of PF4. The amount needed for 50% inhibition varied from 0.2-1.5µg/ml in different donors. Platelet aggregation with epinephrine (2µg/ml) released 4.8µg/ml (±1.7) of PF4 and showed 55% (±22) inhibition of contact activation (n=11). Collagen (5.7µg/ml) caused 4.9µg/ml (±1.3) of PF4 to be released, but the degree of inhibition varied (n=13). The discrepancy between this and the freezing experiment may be due to the " contact-product activator" properties of collagen and the intact platelet membrane. Control experiments performed using indomethacin or EDTA-PRP showed no release of PF4 or inhibition of contact activation.

The effect observed appears to be related directly to the ability of PF4 to bind to negatively charged polysaccharides since PF4 was not found to inhibit activated factor XII or kallikrein. These results indicate another role for PF4 as an inhibitor of contact activation. Together with its known antiheparin properties it indicates that PF4 may be closely involved in the control of haemostasis.