

RELATIONSHIPS BETWEEN FACTOR XII-DEPENDENT SYSTEMS AND THE "PRORENIN"-RENIN SYSTEM IN HUMAN PLASMA. E.A. Wilczynski, A.D. Purdon, and D.H. Osmond. Department of Physiology, University of Toronto, Toronto, Canada.

Treatment of plasma with cold (-4°C , 72 hr), and with trypsin (0.5 mg trypsin/ml plasma), are well established in-vitro techniques used to activate plasma prorenin. Various clotting factor deficiencies have been found to impair the conversion of prorenin to renin in plasma. Studies with factor XII deficient plasma, in which marked reduction in both cold and tryptic activation was seen, led to further studies on the role of clotting factors and other factor XII-dependent systems in prorenin activation. Removal of factors II, VII, IX, and X by adsorption onto BaSO_4 , and subsequent exposure of the residual plasma to cold (-4°C , 48 hr) and trypsin (1 mg/ml), resulted in a decreased capacity for prorenin activation when compared to control plasma, more so in cold than in trypsin-treated plasma. Plasminogen-free plasma responded similarly and, while increased concentrations of trypsin could enhance its prorenin activation to near-normal levels, prolonged cold incubation could not. This suggests that trypsin, added in an appropriate concentration to deficient plasma, may be able to substitute for the missing factor(s), while cold activation is limited by availability of one or more crucial factors. Unmanipulated Fletcher plasma (prekallikrein deficient) has a low level of active renin, and elevated prorenin, symptomatic of a block of prorenin conversion in-vivo. However, cold and tryptic activation were, if anything, relatively greater than normal, especially for trypsin, suggesting that enzymes other than kallikrein are important activators, in-vitro, and can substitute for the missing kallikrein. Thus, neither kallikrein, nor any other single factor studied here, including factor XII, is solely responsible for the activation of plasma prorenin.

Supplementary Abstracts

Factor XII Kallikrein and Kininogen

1064

USE OF CHROMOGENIC PEPTIDE SUBSTRATE ASSAYS IN THE EVALUATION OF SURGICAL TREATMENT OF INTRAABDOMINAL SEPSIS. N. Smith-Erichsen, A.O. Aasen & E. Amundsen. Institute for Surgical Research, Rikshospitalet and Department of Anesthesia, Akershus Central Hospital, Oslo, Norway.

Chromogenic peptide substrate assays were included in the evaluation program of 14 patients having surgical treatment for intraabdominal sepsis.

Six of the patients survived, whereas eight died. The six survivors had a total of 19 operations (1 - 5 operations/patient). The eight fatal cases had a total of 14 operations (1 - 2 operations/patient). Plasma prekallikrein (PKK), functional antikallikrein (KKI), plasminogen (Plg), fast antipiasmin (AP) and functional antithrombin III (AT-III) values were determined using chromogenic peptide substrate assays. Hageman factor levels (HF) were determined using a coagulation assay with a HF deficient plasma. α_2 -Antiplasmin ($\alpha_2\text{AP}$), C^1 -esterase inhibitor (C1INH), α_2 -Macroglobulin ($\alpha_2\text{M}$) and AT III were determined immunochemically.

In both the fatal cases and the survivors PKK, HF, Plg and AT III values were found markedly reduced during sepsis. The reductions of PKK and AT III were significantly more pronounced in the fatal cases than in the survivors. During sepsis the ratio of functional activity versus immunochemical values decreased when compared with normals for $\alpha_2\text{AP}$, C1INH and AT III. Whereas the parameters remained reduced in the fatal cases, gradual increases towards normal values for PKK, HF, Plg and AT III were found within two weeks in the survivors. Clinically and by autopsy all the fatal cases were evaluated to have a persistent intraabdominal septic focus.

It is concluded that chromogenic peptide substrate assays appear to be of great value in evaluating surgical treatment of intraabdominal sepsis.

1065

CHROMOGENIC SUBSTRATES AND ACTIVATED FORMS OF FXII: COMPARISON BETWEEN THE α - AND β -FORMS AND SELECTIVITY WITH REGARD TO PLASMA KALLIKREIN AND FXI_a. L. Aurell, S. Gustavsson and P. Friberger. Kabi AB, Peptide Research, Mölndal, Sweden.

Activated FXII of both the α - (MW 80000) and the β - (MW 28000) form was tested with a large number of chromogenic tri- and tetrapeptides. The activities of the two enzymes correlated well ($r=0.84$, $n=30$). The α -form appeared to have a somewhat higher specificity.

The plasma kallikrein substrate S-2302 and the FX_a substrate S-2222 have earlier been shown to be the commercially available substrates most sensitive to the β -form of activated FXII. The specific activities of both the α - and β -forms are the same with S-2302 in the system tris buffer pH 8.0 and I 0.05 at 37°C . At the enzyme concentration of 4 nmol/l, the change in absorbance per min was 0.02 which is in the suitable measuring range. This concentration is approximately equivalent to fully activated plasma diluted 1:100.

From these findings, it could be confirmed that when plasma prekallikrein is assayed via contact activation with S-2302, there is a definite but small contribution to the hydrolysis of the substrate from activated FXII.

A more selective substrate for activated FXII with regard to plasma kallikrein would be preferable in a plasma assay of FXII. S-2222 has these characteristics. At the substrate concentration of 0.33 mmol/l, the β -form of activated FXII splits S-2222 essentially as efficiently as S-2302 while plasma kallikrein is only 5% as efficient with S-2222 as with S-2302.

Both types of substrates are insensitive to FXI_a at concentrations obtainable in plasma samples suitably diluted for the assaying of FXII and plasma prekallikrein.