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PERSISTENT DECREASED FIBRINOLYTIC ACTIVITY IN CYCLOSPORIN A (CyA) TREATED RENAL ALLOGRAFT RECIPIENTS.
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CyA administration is associated with histological evidence of vascular injury. Since vascular endothelial cell components are known to modify fibrinolytic activity, we have examined fibrinolytic changes in renal allograft recipients on CyA and, in addition, markers of vascular endothelial cell damage: plasma vWFRiCof activity and AT-III activity.

Parameters of fibrinolytic activity have been assessed serially in 21 renal allograft recipients for 1 year post-transplantation by study of the following: euglobulin clot lysis time (ELT), plasminogen activator activity (PAA) by fibrin plate assay, tissue plasminogen activator (t-PA) by ELISA and fast-acting t-PA inhibitor (t-PAI) by chromogenic assay.

From 1 month onwards, fibrinolytic activity (ELTs and fibrin plates) is significantly decreased, $p<0.002$ and $p<0.002$ respectively, compared to normal controls, and this decrease persists at one year post-transplantation. Mean ELTs (mins) and ranges at 1 and 6 months and 1 year are 363 (120-1740), 598 (135-1610) and 538 (80-870) respectively. Mean PAA (% of pooled normal plasma) and ranges at these times are 104 (70-154), 79 (48-118) and 83 (65-108) respectively.

In contrast, serial t-PA levels (%of pooled normal plasma) are significantly increased ($p<0.02$), 428 (86-3686), 297 (81-1130) and 198 (44-637) at 1 and 6 months and 1 year respectively.

These results suggest that the decreased fibrinolytic activity is due to increased levels of t-PA inhibitor, too high to be surpassed by the amount of t-PA released. vWFRiCof activity and AT III activity are similarly increased.

In conclusion, CyA-treated renal allograft recipients show evidence of chronic vascular endothelial damage and a prothrombotic state.

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THE OPPOSING EFFECTS OF BASIC FIBROBLAST GROWTH FACTOR AND TRANSFORMING GROWTH FACTOR BETA ON THE REGULATION OF PLASMINOGEN ACTIVATOR ACTIVITY IN CAPILLARY ENDOTHELIAL CELLS.
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Basic fibroblast growth factor (bFGF), a potent inducer of angiogenesis in vivo, stimulates the production of both the cell-associated and the secreted forms of urokinase- and tissue-type plasminogen activators (PA) in cultured bovine capillary endothelial cells. This stimulation was counteracted by picogram amounts of transforming growth factor beta (TGFb). The stimulatory effect of bFGF was not completely abolished by increasing the amount of TGFb. However, the inhibition by TGFb was greatly enhanced if the cells were pretreated for 1-3 hours with TGFb before addition of bFGF, and the inhibition was almost total, if the preincubation time with TGFb was 6 hours. Sequential changes of serum-containing medium prior to addition of bFGF also blocked the PA stimulatory effect of bFGF. This inhibitory activity of serum was reduced by incubation of the serum with anti-TGFb-IgG. After prolonged incubation of cultures treated simultaneously with bFGF and TGFb, the inhibitory effect of the added bFGF dominated as assayed by PA levels. TGFb did not alter the receptor binding of labeled bFGF, nor did a 6 hour pretreatment with TGFb reduce the amount of bound bFGF. The major difference between effects by bFGF and TGFb was that while bFGF effectively enhanced PA-activity expressed by the cells, TGFb decreased the amounts of both cell-associated and secreted PA activity by decreasing enzyme production and proenzyme activation. Both bFGF and TGFb increased the secretion of the endothelial type 1 plasminogen activator inhibitor (PAI 1). The highest concentration of TGFb is found in platelets, and it is known to be released during clot formation. The suppression of PA production by the endothelium by the release of TGFb should result in a decrease in the fibrinolytic activity and promote clot maintenance. In addition, the rapid stimulation of high levels of PAI 1 secretion from the surrounding capillary cells by platelet released TGFb may further suppress fibrinolysis. The reversability of the TGFb effect and domination of bFGF stimulation may be important in relation to the subsequent onset of clot lysis or angiogenesis leading to thrombus reorganization and wound healing.

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HUMAN MEIZOTHROMBIN 1, ITS ISOLATION AND PROPERTIES.
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Meizothrombin (MT) and meizothrombin des Fragment 1 (MT1) are intermediates in the conversion of prothrombin to α-thrombin (αTH). Due to their transient character, properties of these enzymes are difficult to establish. Isolation of MT1 was achieved by affinity chromatography on D-Phe-Pro-Arginal (FPRal) immobilized on Affi-Gel 10 as originally employed for thrombin purification (Patel et al. Biochim. Biophys. Acta 748, 321 (1983)). Human prethrombin 1 was activated with the purified activator from *Echis carinatus* venom in the presence of Ca^{++}, benzamidine and FPRal gel at pH 7.8. After exhaustive washing the MT1 was eluted with 0.1 M hydroxylamine in 0.15 M Na acetate buffer, pH 5.5. Under these conditions the MT1 is stable and can be stored at -70°C. Upon changing the pH of the preparation to 8.0, complete conversion into αTH occurred at room temperature within 48 hours. Homogeneity of both preparations was demonstrated by PAGE. The K_m and k_c values for MT1 measured on Tos-Gly-Pro-Arg pNA (0.1 M NaCl, 0.01 M TRIS, 0.01 M HEPES, 0.1% PEG, pH 7.8, 25°C) were 15.7 μM and 126 s^{-1}. The kinetic constants for the αTH resulting from autocatalytic degradation of MT1 were indistinguishable from those previously established for αTH obtained by Xa activation i.e. 4.77 μM and 126 s^{-1}. Clotting activity of MT1 was found to be only one fifth as high as that of the resulting αTH (746 u/mg vs. 3900 u/mg as tested using the NIH standard). Inhibition of MT1 by antithrombin III was also much less rapid than αTH and most importantly, it was not affected by high affinity heparin (M_r 20,300). Under conditions of the experiment (0.3 M NaCl, 0.01 M TRIS, 0.01 M HEPES, 2.5 mM EDTA, 0.1% PEG, pH 7.8, 25°C; [ATIII] 100 nM, [E] 10 nM), the pseudo first order rate constants in the absence of heparin were $4.04 \times 10^{-4} s^{-1}$ (MT1) and $1.13 \times 10^{-3} s^{-1}$ (αTH), giving apparent second order rate constants of 4.04×10^{3} and 1.13×10^{4} $M^{-1}s^{-1}$. In the presence of 4.5 nM of heparin the observed first order rate constant for MT1 remained unchanged whereas it increased to $6.241 \times 10^{-3} s^{-1}$ (5.5 fold) for αTH. This apparent lack of an effect of heparin may be of significance *in vivo*.

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IDENTIFICATION OF LYSINES IN HUMAN THROMBIN ESSENTIAL FOR HEPARIN BINDING AND CLOTTING ACTIVITY.
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We are studying human thrombin (IIa) in order to determine the significance of particular amino acid residues critical for interaction with various substrates and cofactors. Previously, we demonstrated the importance of lysyl residues of IIa during interaction with heparin as well as fibrinogen (Griffith, M.J., (1979) J. Biol. Chem. 254:3401; Church, F.C., et al., (1985) J. Biol. Chem. 260:4936). Identification of these essential lysyl residues in IIa has been approached by chemical modification using the amino group-specific reagent pyridoxal-5'-phosphate (PLP) in the presence and absence of heparin. IIa phosphopyridoxylated in the absence of heparin (unprotected) showed approximately 2 mols of PLP incorporated per mol of IIa and had greatly reduced inhibition by antithrombin III (ATIII)-heparin as well as reduced clotting activity. IIa phosphopyridoxylated in the presence of heparin (protected) showed approximately 1 mol of PLP incorporated per mol of IIa and had reduced clotting activity but essentially normal inhibition by ATIII-heparin. Both modified thrombins showed normal inhibition by ATIII and heparin cofactor II in the absence of heparin at 25°C and pH 8.0 with apparent second-order rate constant values ranging from $3\text{-}5 \times 10^{5}$ and $4\text{-}6 \times 10^{4}$ M^{-1} min^{-1}, respectively. In contrast to native IIa, neither protected nor unprotected PLP-IIa derivatives bound to a fibrin monomer-agarose column equilibrated at 25°C with 50 mM Tris-HCl, 50 mM NaCl, pH 7.4. Samples of both modified thrombins were reductively denatured, S-carboxymethylated, and hydrolyzed with trypsin at 37°C. The resultant peptide mixtures were separated using reverse-phase high performance liquid chromatography. Peptides showing a high degree of PLP incorporation were sequenced by automated Edman degradation and the modified lysyl residues were identified in the primary sequence of IIa. In unprotected-IIa, lysyl residues 21, 65, 174 and 252 of the B-chain were modified. In heparin-protected-IIa, only Lys 21 and 65 of the B-chain were modified. These results suggest that Lys 174 and 252 of the B-chain of thrombin are essential for binding to heparin and that Lys 21 and/or 65 are essential for clotting activity.