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DISCREPANCY IN FIBRINOLYTIC RESPONSE TO VARIOUS STIMULI IN HEALTHY MEN. M. Stegnar, I. Keber, D. Keber, N. Vene. University Institute of Gerontology - Internal Clinic Trnovo, Ljubljana, Yugoslavia

Absent or low fibrinolytic response to stimulation is believed to reflect thrombotic tendency. However, it has been observed, that poor response to one stimulus is not always associated with poor response to other stimuli. Therefore, correlation between fibrinolytic responses to various stimuli has been studied in two groups of healthy men. Nine volunteers of the first group were subjected to 20 min upper arm venous occlusion (VO) followed by vasopressin analogue (DDAVP) infusion (0,4 ug/kg body weight in 10 min). In the second group (16 volunteers) VO and physical exercise (EXER, discontinuous test on treadmill up to maximal heart rate) were applied successively in this order. Venous blood was sampled before stimulation and in the 20 min of VO in both groups. The third blood sample was obtained 20 min after start of DDAVP infusion in the first group and immediately after EXER in the second group. Fibrinolytic activity was determined by euglobulin clot lysis time and by specific tissue plasminogen activator (t-PA) activity assay and increase in activity above prestimulation value calculated. No significant correlation between fibrinolytic response to VO and DDAVP was observed in the first group. Also in the second group responses to VO and EXER showed no correlation.

Assay	N	Stimulus	Fibrinolytic response Range	Median	r
t-PA (mIU/ml)	9	VO	74-29 152	1 604	0,47(n.s.*)
		DDAVP	373-11 187	3 864	
	16	VO	2-18 207	1 387	0,30(n.s.)
		EXER	20-11 832	1 414	

* not significant

The observed discrepancy in fibrinolytic responses to various stimuli rise an important question: Which fibrinolytic response is more relevant to development of thrombosis?

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IN VITRO EFFECTS OF RECOMBINANT TISSUE TYPE PLASMINOGEN ACTIVATOR ON FIBRINOLYTIC AND COAGULATION PARAMETERS AND ITS PREVENTION BY SPECIFIC ANTIBODY, D-PHE-PRO-ARG-CH_2Cl AND APROTININ. E.Seifried (1) and P. Tanswell (2). Abteilung Innere Medizin III, Universität Ulm, FRG (1) and Department of Biochemistry, Dr. Karl Thomae GmbH, Biberach/Riß, FRG (2).

Monitoring of systemic effects during rt-PA therapy has shown of depletion of fibrinogen, α_2-antiplasmin, plasminogen and other hemostatic factors. Because in vitro activation of plasminogen may occur between blood collection and freezing and thawing before assaying we analysed the influence of 0,0.2, 2.0 and 10.0 ug rt-PA/ml citrate blood (final conc.) on hemostatic and fibrinolytic parameters and its inhibition by 3 different inhibitors. Addition of rt-PA to citrated whole blood without an inhibitor induced a concentration-dependent depletion of Fbg, Plg, α_2-Apl, α_2-M, C_1-I, α_2-Atrp, a loss of activity of F V, VIII, IX, XIII and alterations of the global coagulation assays. No effect of rt-PA was observed on F II, VII, X, XI, XII, AT III and Protein C. To prevent in vitro fibrinogenolysis 0.1, 0.5 and 1 mg/ml of a polyclonal sheep anti-rt-PA-antibody, 0.3, 1.0 and 10 umol/l PPACK (D-Phe-Pro-Arg-CH_2Cl), 75 and 150 KIU/ml aprotinin (final conc.) and saline as a control were added to pooled citrate blood. All samples containing rt-PA and/or inhibitors and/or saline were incubated for 45 min on ice, centrifuged, aliquotted, snap frozen and stored at -20°C until analysis. Pretreatment of blood samples with anti-rt-PA IgG prevented interferences with all fibrinolytic and most clotting assays in plasma at a dose of 2 ug rt-PA/ml. PPACK was of limited utility in clotting assays, but enabled correct analysis of fibrinolytic assays. Aprotinin was suitable only for a restricted range of both assay types. It is concluded that collection of blood samples on an appropriate antibody may be the most suitable procedure to get correct measurements of in-vivo effects of rt-PA on the hemostatic system in patients undergoing fibrinolytic therapy.

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IDENTIFICATION OF A FIBRINOLYTIC DEFECT IN TWO FAMILIES WITH A TENDENCY TO THROMBOSIS. H.Ostermann (1), S.Koenig (1), H.Pollmann (2), U.Schmitz-Huebner, (1). Department of Medicine (1) and Department of Pediatrics (2) University of Muenster, FRG

We identified two families in whom one member each suffered from deep venous thrombosis and subsequent pulmonary emboli at the age of 15 and 17. We were able to investigate several members of both families with regard to their fibrinolytic system. Blood sampling was done before and after ten minutes of venous occlusion. Parameters measured were euglobulin clot lysis time (ECLT), tissue-type plasminogen activator (t-PA) activity, t-PA concentration and plasminogen activator inhibitor (PAI) activity, besides several other constituents of the coagulation and fibrinolytic system commonly associated with thrombophilia.

In the first family six persons could be evaluated. A prolonged ECLT was found in four probands. Three of them had no measurable t-PA activity after stasis, t-PA concentration after stasis was below the normal range in two and borderline in one of them.

In the second family 13 members could be investigated. Three adults were found to have a prolonged ECLT. Two of these had very low t-PA activity after stasis, with normal increase in t-PA antigen. Their PAI was increased above the normal range. Six children showed no measurable PAI and increased levels of t-PA activity before stasis. After stasis four children had a prolonged ECLT. Two of them had low t-PA antigen levels, while all of them showed normal t-PA activity increases.

These findings suggest, that there may be hereditary defects related to activators and inhibitors of the fibrinolytic system in the investigated families. These could possibly be responsible for the occurence of venous thrombosis early in life. However, results in the second family show that not all of the prolonged ECLT values can be explained by changes of t-PA and PAI.

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THE EFFECTS OF PHYSIOLOGICAL CONCENTRATIONS OF VASOPRESSIN ON COMPONENTS OF THE FIBRINOLYTIC PATHWAY. H. Hariman, J.R. Hughes, P.J. Grant and J.A. Davies. University Department of Medicine, The General Infirmary, Leeds. LS1 3EX, UK.

Evidence from studies in man suggests that vasopressin (aVP) at physiological concentrations activates the coagulation pathway, increases plasminogen activator activity and may have a role in the regulation of haemostasis under conditions of physical stress. Infusion of aVP in normal subjects increases plasma factor VIII concentrations and shortens the euglobulin clot lysis time (ECLT), but the mechanisms involved in these changes and their haemostatic significance are unclear. The aims of this study were to investigate the effects of aVP on the fibrinolytic pathway and to evaluate whether thrombin or plasmin are generated in vivo by aVP. After 30 min 0.9% saline infusion, vasopressin (20iu in 250ml 0.9% saline) was infused at 2.0 u/h for 1h in 9 normal subjects to achieve plasma aVP concentrations comparable to those attained during stress. Venous blood samples were taken before saline infusion (time 0) and every 30 min for 2h for assay of aVP, activated partial thromboplastin time (APTT), fibrinopeptide A (FPA), FPA generation time, FPBß15-42 ECLT, tissue-type plasminogen activator (t-PA) and t-PA inhibition. Plasma aVP rose from 0.5 pg/ml at time 0 to (median) 70.7 pg/ml at 90 min. The APTT shortened from 43.8 ± 1.9 to 34.4 ± 1.6 (SEM) seconds ($p < 0.001$) at 90 min. Plasma FPA and the FPA generation time remained unchanged ($p > 0.05$). Plasminogen activator activity rose from 36.4 ± 15.2 to 587.5 ± 206.6 units ($p < 0.005$), t-PA increased from 229.8 ± 20.4 to 1107.4 ± 224.1 ml.U/ml ($p < 0.005$) and t-PA inhibition fell from 7.9 ± 1.1 to 3.9 ± 0.9 I.U/ml ($p < 0.05$) in response to the aVP infusion. FPBß15-42 increased from a baseline value of 1.7 ± 0.4 to 2.2 ± 0.7 pmol/ml after 90 min ($p < 0.05$). The results suggest the effects of aVP on fibrinolysis are due to an increase in t-PA and decrease in t-PA inhibition. The increase in FPBß15-42 with no change in FPA supports the hypothesis that plasmin was generated by non-fibrin dependent pathways.