

COMPARISON BETWEEN VASCULAR PLASMINOGEN ACTIVATOR ACTIVITY AND BLOOD FLOW IN THE RENAL CORTEX AFTER INDUCTION OF HEMORRHAGIC HYPOTENSION AND INFUSION OF INDOMETHACIN OR SARALASIN IN PIGS. A.Smokovitis, M.Maier, and B.R.Binder, Dept. of Med.Physiol. Univ. of Vienna, Vienna, Austria

Patterns of vascular plasminogen activator activity and blood flow were compared in the renal cortex of pigs after induction of hemorrhagic hypotension (1ml blood loss per kg and minute until the death of the animal) and infusion of indomethacin (prostaglandin synthetase inhibitor) or saralasin (competitive inhibitor of angiotensin II). Blood flow was measured by radioactive labelled microspheres. Pigs infused with indomethacin or saralasin during hemorrhage showed by the end of the experiments (mean arterial blood pressure 40-50 mmHg) almost the same decrease in the outer (65-70%) and inner (55-65%) renal cortical blood flow (RCBF) compared to the RCBF values before hemorrhage. However, only in saralasin treated pigs a change in the plasminogen activator activity (PAA) was induced (30% decrease in the inner and 45% in the outer renal cortex). In pigs subjected to hemorrhage and infusion of the corresponding vehicles for the inhibitors the vascular PAA in the renal cortex remained unchanged despite a marked change in RCBF (decrease to 4-7% by the end of the experiments). In indomethacin treated pigs without bleeding RCBF was diminished to 80% of the values before infusion of indomethacin until the end of the experiment (100 minutes infusion of indomethacin); no change in the vascular PAA was seen. In saralasin treated pigs without bleeding RCBF increased initially (20%) and returned to control values until the end of the experiment; the vascular PAA in the renal cortex showed a 15% decrease. Changes in plasmin inhibitor activity in the renal cortex were not noted.

Under the conditions of these experiments no correlation could be found between induced changes in RCBF and vascular PAA in the renal cortex. The vascular fibrinolytic response to saralasin should be due to an indirect effect of saralasin independent of the changes in RCBF induced by this agent.

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ACTION OF FOY, A POLYVALENT SYNTHETIC INHIBITOR ON COAGULATION AND FIBRINOLYSIS. A.Stemberger, S.Haas, R.Stöhr, G.Blümel. Institute of Experimental Surgery of the Technical University Munich, FRG

Foy(S 983) ,a low molecular synthetic protease inhibitor is discussed as a potential drug in acute pancreatitis and in traumatic haemorrhagic shock.Action on coagulation was tested using global clotting tests like aPTT and prothrombin time. In addition, the inhibitory effect was elucidated with chromogenic substrates on the X a- and thrombin level after exogenous or endogenous activation. This study was performed in comparison with aprotinin (Trasylol^R) based on the assumption that 50 KIE aprotinin \approx 0.1 mg Foy.

Effect on fibrinolysis was tested after streptokinase (Sk) or urokinase activation.Due to the kallikrein,Xa and thrombin inhibition Foy showed a dose dependent strong effect on the endogenous and exogenous pathway in contrast to the weak effect of aprotinin on the endogenous system via kallikrein only. Plasmin inhibition of the two drugs were similar. However Foy interfered with the Sk-plasminogen activation and is an inhibitor of urokinase.Both drugs showed a strong inhibition on proteases harvested from the secretion of fistulas of the intestinal tract which are often responsible for disturbances in wound healing.

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FACTORS INFLUENCING THE STRUCTURE OF TERMINAL PLASMIN DEGRADATION PRODUCTS OF HUMAN FIBRINOGEN AND FIBRIN. W. Nieuwenhuizen, A. Vermond and F. Haverkate. Gaubius Institute, Health Research Organization TNO, Leiden, The Netherlands.

Experiments have been carried out with fibrinogen and with purified degradation products of fibrinogen and fibrin which demonstrate that the structure of D fragments obtained after prolonged plasmin digestion is influenced by several factors in the media.

The previously described protective effect of calcium ions on the γ -chain carboxy-terminals of fibrinogen against plasmin attack is rather independent of the composition of the media (e.g., also observed in 2 M urea and/or high plasmin activities).

Several compounds such as EDTA, EGTA, citrate and iminodiacetic acid appear to have a separate effect, which is best observed at low plasmin concentrations and in the absence of Ca^{2+} . Under these conditions, these compounds appear to make the γ -chain carboxy-terminal ends of the D- and D-dimer fragments more susceptible to plasmin digestion.

Finally, as demonstrated by experiments with purified D:E complexes from fibrinogen and with whole fibrinogen digests, the E-moiety of the D:E complexes appears to be capable of protecting the D-moiety against low plasmin concentrations also in the absence of calcium ions.

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THE ESTIMATION OF α_2 -ANTIPLASMIN BY MEANS OF SYNTHETIC SUBSTRATES: KINETIC ANALYSIS AND OPTIMIZATION OF THE METHOD; G. Dooijewaard and C. Klufft. Gaubius Institute TNO, Leiden, The Netherlands

Generally the concentration of an enzyme inhibitor in plasma is estimated as follows: plasma, in decreasing dilutions, is incubated with a fixed amount of enzyme. When the inhibitor has been irreversibly converted to its enzyme-inhibitor complex, the excess of enzyme is estimated as the initial rate (v) of its activity with a synthetic substrate. The plot of v against plasma dilution results in a straight line which intercepts the abscissa at the point of equivalency.

Contrary to the above method, for the estimation of α_2 -antiplasmin (A) in plasma, the excess of plasmin (Pl) is measured immediately after the attainment of a fast equilibrium between Pl, A and the reversible complex PlA (equilibrium constant K_i). Since this is attained almost two orders of magnitude faster than the irreversible reactions of Pl with inhibitors in plasma other than A, the assay, thus, becomes more specific for A.

The results of the kinetic analysis are: 1. The involvement of the reversible equilibrium causes the plot of v against plasma dilution to be a hyperbola instead of a straight line. The hyperbolic shape of the plot becomes more pronounced at higher ratios of K_i over Pl concentration. Moreover, the synthetic substrate (S) shifts the equilibrium to dissociation, thereby apparently increasing the K_i by a factor of $(1 + S/K_i)$. 2. Pl inhibitors in plasma other than A, interfere relatively more at low plasma dilutions. Because, coincidentally, this interference makes the hyperbola resemble a straight line, the contribution of the other inhibitors is largely overlooked.

It is concluded that the usual assay may have its merit as a routine bioassay, provided the conditions are chosen carefully. As an alternative approach it is recommended to titrate a fixed amount of plasma with increasing concentrations of Pl and to evaluate the initial velocities in a Scatchard plot. In this way a more reliable point of equivalency and in addition the K_i value can be obtained.