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PLASMINOGEN ACTIVATOR (PA) ACTIVITY IN METASTATIC CELLS: AN AMIDOLYTIC APPROACH. D. Coen, A. Bini, G. Balconi, F. Delaini, L. Mussoni and M.B. Donati. Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy

It has been proposed that fibrinolytic activity can play an important role in the process of metastasis formation. Nevertheless, it is not yet clear in which phase of the tumor growth and dissemination this activity is involved. We measured the fibrinolytic activity of cells from primary tumor and metastatic nodules of 3LL, an i.m. implanted murine tumor which selectively metastasizes to the lungs. Tumor cells have been studied both immediately after mechanical disruption of tumor tissue and after in vitro culturing to confluence. Their P.A. activity was tested by an amidolytic assay in which cells were incubated with purified plasminogen (3CU/ml) and 4mM S-2251 (Kabi Diagnostica, Stockholm, Sweden), a plasmin specific chromogenic substrate. After 3 hour incubation at 37°C, the reaction was stopped with acetic acid and absorbance read at 405 nm.
Cells from the primary tumor and metastatic nodules showed a similar fibrinolytic activity, which was in both cases inincreased 3 to 4 fold in cell extracts obtained after preincubation with TRITON X-100. A dose-response curve plotted with increasing urokinase concentrations showed a parallel course. This data suggests that, in the 3LL model, PA activity is not one of the properties characterizing the selection of metastatic cells.
On the other hand, cultured cells presented consistently higher levels of PA than their native counterparts, suggesting that adhesion of cells in culture may stimulate PA production or, alternatively, that cultured cells are a selected population in comparison to the overall number of native cells.

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ANTICOAGULANT AND FIBRINOLYTIC PROPERTIES OF THE SALIVARY GLAND PROTEINS FROM THE LEECH HAEMENTERIA GHILIANII. Andrei Z. Budzynski, Stephanie A. Olexa, Barbara S. Brizuela, Roy T. Sawyer, and Gunther S. Stent. Thrombosis Research Center, Temple University H.S.C., Philadelphia,PA.

Blood-sucking animals, such as leeches, mosquitoes, and vampire bats contain in their saliva agents that prevent the host blood from coagulating as it is being withdrawn. The work reported here was carried out to elucidate the nature of the anticoagulant found in the saliva of the giant leech Haementeria ghilianii, native to French Guyana. The two bilateral pairs of salivary glands of H. ghilianii are located at the proboscus base and encapsulated by a thin membrane. The anterior glands are 20 mm long and contain predominantly the cells of large size; the posterior glands are 5 mm long and contain the cells of very small size. Both salivary glands of the leech contain an anticoagulant that not only inhibits the clotting of human and bovine plasma, but also dissolves previously formed fibrin clots. This anticoagulant activity is attributable to an enzyme, for which the name hementin is proposed. Hementin catalyzes the proteolytic degradation of fibrinogen and fibrin, even in the presence of the inhibitors of proteases occurring in human plasma. The enzyme has the same affinity for human fibrinogen and fibrin. It cleaves human fibrinogen to yield characteristic fragments of high molecular weight that are different from Fragments X,Y,D and E resulting from the digestion of fibrinogen by plasmin. Both the anterior and posterior salivary glands contain hementin. Fractionation of salivary gland homogenates by differential ultracentrifugation showed that hementin is found entirely in the cytosol fraction. The salivary extracts do not contain any appreciable amounts of an activator of human plasminogen or an inhibitor of human or bovine thrombin. thus, Haementeria ghilianii prevents coagulation of its host's blood through a fibrinogenolytic mechanism that is entirely different from that of hirudin, a thrombin inactivating polypeptide present in the saliva of another leech, Hirudo medicinalis.

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DISSOCIATION OF APROTININ-HUMAN PLASMIN COMPLEX BY FIBRIN. E.G. Vairel, M. Thely and H. Brouty-Boye. Institut Choay, Paris (France)

Aprotinin inhibits plasmin by forming an inactive complex which has a binding force of 3.10^8.

When the complex, in a 0.15M buffer solution at pH 7.4, is percolated through a column of human fibrin balanced with the same buffer, all of the aprotinin appears promptly in the eluent and the fibrin column dissolves progressively from the top. If the buffer is replaced with a 0.15M epsilon amino-caproic acid solution as soon as lysis begins, the fibrinolysis is stopped and plasmin is eluted.

If citrated human plasmin is used in a 1:1 solution with buffer, the same events take place but over a longer period of time ; the aprotinin appears slowly in the eluent and fibrinolysis begins later.

It thus appears that fibrin is able to dissociate the aprotinin-plasmin complex, probably by forming a short-lived ternary complex. The complex binds to fibrin even in a plasma medium, and fibrinolysis occurs even with an excess of plasmic inhibitors in the medium.

Fibrinogen does not possess this property of fibrin. The complex can be detected in the circulating blood several hours after intravenous administration to rabbits.

This property of fibrin suggests a possible use of the aprotinin-plasmin complex as specific fibrinolytic treatment.

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TOLERANCY OF ANIMAL FIBRINOLYTIC SYSTEM TO EXOGENOUS PLASMINOGEN ACTIVATOR. G.V.Andreenko, E.E.Shimonaeva, L.V.Lyutova. Laboratory of Enzymatic Fibrinolysis, Moscow State University, Moscow, USSR

Our previous studies showed that intravenous injection of tissue plasminogen activator (TPA) from pig heart to albino rats causes lysis of artificial clots in v.jugularis. This is concomitant with a short-term increase in the fibrinolytic activity (FA) of the euglobulin fraction of blood plasma. The other components of the fibrinolytic system remain thereby unchanged. We tested the state of the fibrinolytic system at increasing concentrations of TPA and under varying conditions. A repeated (2-fold) injection of TPA did not further increase FA. When TPA was injected 4 times, the antiactivator and the level of TPA in the plasma of test and control animals decreased. This can be due to exhaustion of endogenous plasminogen activator as a stress response to repeated fixation of animals for intravenous injection of TPA. Injection of the same amount of TPA for 5 days caused no significant changes in FA. After a single injection of a 5-fold amount of TPA the changes were more noticeable; fibrinogen concentration was slightly decreased, whereas plasma recalcification time was increased. Thus, administration of exogenous TPA causing clot lysis has no prolonged effect on FA, which can be either due to its rapid release from the blood stream or to its binding to blood proteins.