## 1592

PATHOGENIC BACTERIA HAVE HIGH AFFINITY RECEPTORS SPECIFIC FOR PLASMIN. T.A. Broeseker, M.D.P.Boyle, and R. Lottenberg. University of Florida College of Medicine, Gainesville, FL, U.S.A.

Binding of the key fibrinolytic enzyme, plasmin, to certain pathogenic group A streptococci was studied. In these experiments the ability of a group A streptococcal strain, 64/14, to bind either $^{125}$I-human plasminogen or the same label following activation with urokinase was measured. It was found that this strain bound <10% of the labeled plasminogen but >70% of labeled plasmin. This property distinguishes the plasmin receptor from streptokinase. These bacteria did not express a common serine protease receptor/inhibitor since they failed to bind labeled trypsin or urokinase. Maximal binding of plasmin occurred between pH 6.0 and 8.0 and in the ionic strength range of 50-200 mM salt. The Kd of plasmin binding to bacteria was approximately $10^{-10}$ M at pH 7.4 in 150 mM salt. This was determined by a non-linear least squares analysis of equilibrium binding data. Binding was reversibly inhibited by either epsilon aminocaproic acid ($I_{50}$ of 0.2 mM) or lysine ($I_{50}$ of 3.0 mM) suggesting the involvement of the high affinity lysine binding site of plasmin in its binding to bacteria. Bacterial bound plasmin retains its enzymatic activity, being capable of cleaving chromogenic substrates and solubilizing a fibrin clot. The bacterial bound enzyme activity was inhibited by the low molecular weight inhibitors aprotinin and phe-pro-arg chloromethyl ketone but not by alpha-2 plasmin inhibitor. The ability of bacteria to acquire membrane associated proteolytic activity which cannot be physiologically inhibited may contribute to their tissue invasive properties.

## 1593

TISSUE PLASMINOGEN ACTIVATOR (T-PA) PRODUCTION BY HUMAN EMBRYONIC FIBROBLASTS, IMR-90, STIMULATED BY PROTEOSE PEPTONE. Y. Itagaki, A. Suzuki and K. Higashio. Research Institute of Life Science, Snow Brand Milk Products, Co. Ltd., Ishibashi, Tochigi Japan.

In order to study the mechanisms by which t-PA production by IMR-90 cells are induced, lactalbumin hydrolysates, yeast extracts, and peptones were tested for their ability to induce t-PA production by IMR-90 cells. IMR-90 cells were grown to confluency in Dulbecco's modified Eagle's medium(DMEM) supplemented with 10% fetal calf serum at 37°C in 5% $CO_2$ in air. And the cells were maintained in serum free medium containing 1% of each additive. The plasminogen activator activity was determined by fibrin plate method, using urokinase or t-PA from WHO as a standard. It was found that proteose peptone (Difco) and neopeptone (Difco) strongly induced the t-PA production by IMR-90 cells. The t-PA production in DMEM containing 1% proteose peptone reached approx. 200IU/ml after incubation at 37°C for 6 days and was from twenty to fifty times higher than that in DMEM only (control medium). The t-PA production by IMR-90 cells stimulated by proteose peptone was strongly inhibited by RNA synthesis inhibitor(actinomycin D) or protein synthesis inhibitor (cycloheximide). Hence, t-PA production by IMR-90 cells stimulated by proteose peptone was mediated by de novo synthesis. Chelating reagent (EGTA), $Ca^{2+}$ entry blocker (verapamil), inhibitor of phospholipase $A_2$ (quinacrine) and inhibitor of lipoxygenase (NDGA) strongly inhibited the t-PA production by IMR-90 cells stimulated by proteose peptone. Inhibitor of cyclooxygenase (indomethacin) was inert. On the contrary, activators of phospholipase $A_2$($Ca^{2+}$,melittin) and hydroxy-unsaturated fatty acid (5-HETE) derived from arachidonic acid by lipoxygenase strongly enhanced t-PA production by IMR-90 cells stimulated by proteose peptone. These results suggest that the t-PA production by IMR-90 cells stimulated by proteose peptone is mediated by arachidonate cascade involving the following pathway; (1) proteose peptone stimulates the membrane of IMR-90, (2) this stimulus causes $Ca^{2+}$ influx, (3) $Ca^{2+}$ ion activates phopholipase $A_2$, (4) activated phospholipase $A_2$ liberates arachidonic acid from phospholipids in cell membrane and (5) lipoxygenase converts arachidonic acid into the hydroxy-unsaturated fatty acid.

## 1594

PURIFICATION AND CHARACTERIZATION OF TISSUE PLASMINOGEN ACTIVATOR PRODUCED BY IMR-90 CELLS. T. Morinaga, Y. Itagaki, A. Suzuki, H. Yasuda and K. Higashio. Research Institute of Life Science, Snow Brand Milk Products, Co. Ltd., Ishibashi, Tochigi, Japan.

Tissue plasminogen activator ( t-PA ) produced by IMR-90 ( human embryonic fibroblast ) cells cultured in the serum-free medium ( DMEM ) containing 1% proteose peptone and 1.8 - 3.6mM $CaCl_2$ was purified by the procedure consisted of ultrafiltration,immunoadsorption chromatography, HPLC and lysine-Sepharose chromatography. The yield of t-PA from the culture broth was approximately 47%. The purified t-PA migrated as a single band on SDS-polyacrylamide gels. The molecular weight of the t-PA was estimated to be 66,000 by SDS-polyacrylamide gel electrophoresis and 69,000 by gel filtration method. Purified t-PA had a specific activity of $36 \times 10^4$ IU/mg protein by fiblin plate method or $54 - 56 \times 10^4$ IU/mg protein by clot lysis method using t-PA obtained from WHO as a standard. The amino acid composition of fibroblast t-PA was very similar to those of melanoma t-PA and uterine t-PA. Isoelectric point of fibroblast t-PA ranged from 5.7 to 8.2. The t-PA had twice as much affinity for fiblin as did high molecular weight urokinase ( UK ). Both t-PA and UK had optimum temperature at 41°C and optimum pH between 8.0 - 9.0. The polyclonal and monoclonal antibodies raised against t-PA quenched t-PA activity but had no effect on UK activity. The inhibitors of serine proteases, difluorophosphate and gabexate mesilate, strongly inhibited the activities of fibroblast t-PA and UK. The nucleotide sequence analysis of the t-PA cDNA isolated from the cDNA library prepared from IMR-90 mRNA revealed the nucleotide changes at two positions in the coding region as compared to that of melanoma t-PA cDNA. Neither of the charges replaced the coded amino acid. The N-terminal amino acid of fibroblast t-PA was determined to be valine, indicatig the structural similarity of fibroblast t-PA to uterine t-PA.

## 1595

SINGLE-CHAIN UROKINASE TYPE PLASMINOGEN ACTIVATOR (SCU-PA) FROM HT-1080 HUMAN FIBROSARCOMA CELLS IS A GENUINE PROENZYME. L.Skriver(1), L.C. Petersen(2), L.R. Lund(1), L.S. Nielsen(1) and K. Dano(1). Finsen Laboratory, Rigshospitalet, Copenhagen(1) and NOVO Research Institute, Bagsvaerd, Denmark(2)

U-PA is released from many cells as a single polypeptide chain (scu-PA) that is converted into its active two-chain form (tcu-PA) by limited proteolysis with plasmin. There is general agreement that scu-PA has an extremely low amidolytic activity, but different oppinions exist, as to whether scu-PA itself can activate plasminogen. We have reinvestigated the plasminogen activating activity of our scu-PA preparations by means of a direct [125]I-plasminogen conversion assay and two amidolytic assays for plasmin and u-PA activity. In the [125]I-plasminogen conversion assay in the presence of bovine pancreatic trypsin inhibitor (BPTI) the subsequent plasmin catalyzed conversion of scu-PA is blocked while the plasminogen activation is unaffected. In this assay with 300 nM Glu-plasminogen and 15 µM BPTI, 40 nM scu-PA caused a low but significant plasminogen conversion, which could be fully inhibited by pretreatment of scu-PA with diisopropylfluorophosphate (DFP). DFP-treated scu-PA was convertible to fully active tcu-PA. Rates of plasminogen activation in this type of assay for scu-PA activity was at least 400 fold slower than that measured for tcu-PA. A coupled amidolytic assay with Lys-plasminogen, scu-PA or tcu-PA, BPTI, and the high affinity plasmin substrate H-D-Val-Phe-LyspNA (S2390) was performed under conditions that ensures a low steady state concentration of free plasmin. In this assay the initial rate of Lys-plasminogen activation by DFP-treated scu-PA was at least 250 fold slower than that measured for tcu-PA. Finally, u-PA activity was measured in an assay with the chromogenic substrate <Glu-Gly-ArgpNA (S2444) (0.8mM) in the presence of highly purified Glu-plasminogen (300nM) and DFP-treated scu-PA (2nM) in the absence of BPTI. Within the initial 15 min of incubation no detectable hydrolysis of S2444 occurred. Addition of tcu-PA (2pM) or plasmin (0.1nM) to the scu-PA/Glu-plasminogen mixture caused a significant reduction of the lag period before onset of the cascade reaction leading to scu-PA conversion and subsequent hydrolysis of S2444. We conclude that the low rates of plasminogen activation measured in these assays by scu-PA might be accounted for by the presence of trace amounts of tcu-PA in the scu-PA preparations, and that scu-PA meets the requirements for a genuine proenzyme