

**Monday, July 13, 1981**

## Oral Presentations

### Fibrinolysis – I

08:00–09:30 h

### Fibrinolysis – II

09:45–11:00 h

**Dominion Ballroom North**

ACTIVATION AND "INACTIVATION" OF A PLASMINOGEN PROACTIVATOR (PREUROKINASE) IN HUMAN TISSUE CULTURE MEDIA. G. Wijngaards and M.B. Bernik. Gaubius Institute TNO, Leiden, The Netherlands, and Northwestern University Medical School, Chicago, Illinois 60611, U.S.A.

Besides active plasminogen activators, the synthesis of a proactivator (preurokinase) has been reported in cell and tissue cultures. It is suggested that slow activation of the proactivator in the culture medium occurs by an unknown proteolytic mechanism. Rapid activation can be obtained by incubation with plasmin or trypsin, whereas incubation with thrombin results in a preurokinase which has lost its capacity to be activated. These studies were performed with the fibrin plate method (FPM) which in itself involves further exposure of preurokinase to plasmin over a 16 to 20 hr period.

In the present study the effect of incubation of some culture media with plasmin and thrombin, on the activation of proactivator, was measured using the clot lysis time method (CLTM) and an immediate assay with the synthetic substrate S-2322.

The results with the CLTM showed that incubation of particular culture media with plasmin increases the activator activity immunologically related to urokinase. Incubation with thrombin was without effect, but when followed by incubation with plasmin a decreased level of activatable preurokinase was found. The decrease correlated positively with the thrombin concentration and incubation time and negatively with previous treatment of thrombin with antithrombin III. The S-2322 method showed very similar results, while comparison of the results of both CLTM and S-2322 method with those of the FPM revealed only small differences. Results indicate that preurokinase is hardly activated by plasmin in fibrinolytic assays, not even during the long incubation time of the FPM. Accordingly, it seems unlikely that there will be a positive feedback in thrombolysis as a consequence of activation of preurokinase by plasmin. (Supported, in part, by NATO Research grant RG 018.80).

## 0014

08:30 h

FURTHER CHARACTERIZATION OF SK-POTENTIATOR OF PLASMINOGEN. A. Takada, K. Mochizuki and Y. Takada. Department of Physiology, Hamamatsu University, School of Medicine, Hamamatsu, Japan

Streptokinase (SK) forms a complex with human plasminogen (plg) or plasmin, and the resulting complex (SK-activator) functions to convert plg to plasmin. We have indicated that human plasma contains a factor (SK-potentiator) which potentiates the capacity of SK to activate human plg. SK-potentiator has a molecular weight of 240,000, and composed of  $\beta$  and  $\gamma$ -chains of fibrinogen,  $\alpha$ -chain being degraded. SK-potentiator crossreacts with anti-FDP-Y fragment. Immunodiffusion shows that SK-potentiator has an antigenic determinant in common with both FDP-Y and fibrinogen, and the other determinant not in common with fibrinogen but FDP-Y. Early FgDP potentiates SK-activator activity as much as SK-potentiator, but further degraded FgDP potentiates less than fibrinogen which still enhances SK-activator activity. The addition of thrombin to FgDP or SK-potentiator enhances SK-activator activity more than SK-potentiator. Thus removal of fibrinopeptides from FgDP or SK-potentiator results in better potentiator activity. When tranexamic acid (1 mM) was added to the mixture of Glu-plg and UK, the activation of Glu-plg was enhanced, but tranexamic acid (1 mM) added to SK-activator caused a decrease in SK-activator activity. The addition of fibrinogen or SK-potentiator to the mixture of tranexamic acid and SK-activator prevented the decrease of SK-activator activity to some extent, which may indicate that SK-potentiator competes with tranexamic acid for lysine binding sites (LBS) of plg and SK-potentiator forms a complex with SK-activator in spite of the presence of tranexamic acid. It is proposed that SK-potentiator binds with LBS of plg part of SK-activator and SK combines with light chain part of plg, the resulting SK-plg-potentiator complex being the better activator than SK-plg or SK-plasmin complex.

## 0013

08:15 h

EFFECT OF NEURAMINIDASE ON PLASMIN FORMATION BY UROKINASE AND THE VASCULAR PLASMINOGEN ACTIVATOR. M. Geiger and B.R. Binder, Dept. of Medical Physiology, University of Vienna, Vienna, Austria.

It was the aim of the present study to determine whether sialic acid residues in the activator or substrate molecule play a role in the activation of plasminogen to plasmin by the vascular plasminogen activator (VPA) and urokinase (UK).

Using a purified test system with UK ( $M_r=31,000$ , human urine), VPA ( $M_r=70,000$ , cadaver vessel eluates) as activators, Glu-plasminogen as substrate, and pretreatment with neuraminidase (C.perfringens, 1.5IU/mg) it could be shown by quantification of the formed plasmin with S-2251 as substrate that neuraminidase treatment enhances plasmin formation by UK as well as by VPA. Using Sepharose-bound neuraminidase (C.perfringens, 20-30U/g Sepharose) it could be shown that neuraminidase affects the substrate in a dose and time dependent fashion, while the activity of the activators seems to remain unchanged. Treatment of plasminogen with neuraminidase in a concentration of 2.5 mU per  $\mu$ mole plasminogen for 90 min. at 37°C increased the plasmin formation by VPA by about 50% which was the same as for UK.

Kinetic analysis of plasmin formation from neuraminidase treated plasminogen by UK and VPA suggests increased binding of the substrate to the activators (unchanged  $V_{max}$  and decreased  $K_M$ ). The enhancing effect of fibrin on plasmin formation by VPA seems to remain unaltered by neuraminidase treatment of the plasminogen.

These data suggest that, as in the case of other protease substrates, removal of sialic acid residues results in increased cleavage of the substrate, i.e. enhanced plasminogen activation, regardless of the type of activator.