ELECTROPHORETIC INVESTIGATION OF HUMAN TISSUE PLASMINOGEN ACTIVATORS

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Experiments with plasminogen activators isolated from several human tissues failed to reveal any difference in their electrophoretic mobility. However, such a difference in the case of tissue activators and urokinase appeared at pH ≥8.0. The rate of migration of urokinase toward the cathode was greater than that of the tissue activators.

Investigations have shown that tissue plasminogen activator is present in most tissues, mainly in the lysosomes of cells and the microsomes of endothelial cells of the blood vessel walls [7]. Fluctuations in the content of activator in different tissues and different individuals reveal no direct correlation between the content of the tissue activator and the functional properties of the organ [2]. On electrophoresis of a purified preparation of tissue activator, three bands are found, and only the fastest band possesses fibrinolytic activity [8]. At least two activators are present in the tissues [4]: one is analogous to the blood activator liberated on injection of vasodilator drugs; the other is secreted from the intima of the vessel wall only under the influence of substances rupturing ionic and hydrogen bonds, such as 0.5 M potassium thiocyanate. Ottolander et al. [9] consider that the concept of "tissue activator" is a collective term covering several substances.

The properties of activators from several human tissues and urine were studied in order to identify and detect the isoenzymes.

EXPERIMENTAL METHOD

Material for investigation was taken from 10 cadavers. The tissues were taken not later than 24 h after death. The activators were isolated immediately or after the tissues had been kept at −25°C by the method of Astrup and Albrechtsen [3]. Electrophoresis was carried out in 1% Difco agar gel in 0.02 M phosphate buffer, pH 7.0-9.5, containing potassium thiocyanate solution, at room temperature and using the UÉF-1 chamber on glass slabs measuring 9 × 12 cm. The potential gradient was 5 V/cm, the current 45 mA, and the duration of electrophoresis from 2 to 4.5 h. After electrophoresis the surface of the gel was covered with a second thin layer of fibrin-agar gel containing plasminogen.

The technique of preparation of preparation of the fibrin agar was as follows: 1.5% agar-agar gel in veronal buffer, pH 7.8, was heated until it had completed liquefied and was then cooled to 45°C. A solution of thrombin (Kaunas Bacterial Preparations Factory) containing 100 units to 1 ml physiological saline was made up beforehand. Three parts of the liquefied agar gel were mixed with one part 0.6% fibrinogen solution not purified from plasminogen, in veronal buffer, pH 7.8. Next, 1 ml of the thrombin solution was quickly added and stirred. The resulting mixture was used for the added layer. Preparations of bovine fibrinogen [6] or batches of fibrinogen produced by the Kaunas Bacterial Preparation Factory are suitable for this type of work.

The surface of the fibrin-agar gel gas covered with chromatography paper to accelerate diffusion of the activator from the bottom layer into the top (fibrin agar). The plates were dried at 37°C, fixed with 2% acetic acid solution, and stained for protein with amido black 10B. For one analysis 0.01 ml of activator isolated by the method of Astrup and Albrechtsen [3] was used. Urokinase was used for comparison [1].

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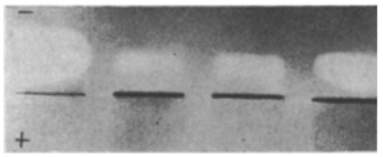

Fig. 1

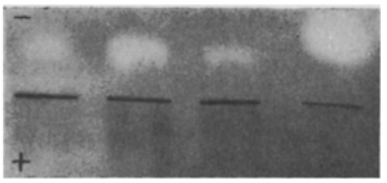

Fig. 2

Fig. 1. Electrophoresis (from left to right) of urokinase and of plasminogen activators from the kidney, aorta, and lymph glands for a period of 4 h at pH 9.0. Light zones correspond to localizations of enzymes.

Fig. 2. Electrophoresis of plasminogen activators (from left to right) of myometrium, endometrium, and adrenals and of urokinase for 4.5 h at pH 8.0. Light zones correspond to localizations of enzymes.

EXPERIMENTAL RESULTS

The properties of activators isolated from tissues of the lung, brain, heart, aortic wall, lymph glands, adrenal and prostate glands, perimetrium, myometrium, and endometrium were studied. Tissue activators from the kidney and its pelvis, the ureter, and the urinary bladder also were used as sources for isolation of urokinase. Parallel electrophoresis of eight activators isolated from different tissues, of urokinase and of human blood serum was carried out simultaneously on plates measuring 9×12 cm.

The rate of migration of the tissue activators and urokinase relative to the serum fractions migrating parallel with them, and also the magnitude of their activity depended not only on the pH of the buffer, but also on the KCNS added to the system. With an increase in the KCNS concentration to 0.1 M, the activity and rate of migration of the enzymes toward the cathode were increased. The optimal value of the hydrogen ion concentration for detection of tissue activators by electrophoresis is 9.0, for urokinase pH 7.8.

In the experiments with activators isolated from the above-mentioned tissue, no difference could be found between their relative mobilities by electrophoresis at pH 7.0-9.0 (Fig. 1). However, a difference between the electrophoretic mobilities of the tissue activators and urokinase did appear at pH 8.0 (Fig. 2). The rate of migration of urokinase toward the cathode was greater than that of the tissue activators.

It can be concluded from these results that, despite differences in the ability of the tissues to activate plasminogen, no difference can be found between their electrophoretic properties. This suggests that different tissues contain the same activator, but in different amounts. However, there is no doubt whatever that the plasminogen activator excreted in the urine differs substantially from the tissue activators. The view [5] that urokinase is a filtration product of the tissue activators, circulating in the blood, into the urine is thus unconvincing. Urokinase is evidently an independent enzyme which differs from the tissue plasminogen activators.

Since all activators migrated as a single band at pH values from 7.0 to 9.5, this suggests that different tissues contain basically the same highly active enzyme which converts plasminogen into plasmin. Bearing in mind the relatively low sensitivity of the method used in the present experiments to detect plasminogen activators, the possibility cannot be ruled out that tissue plasminogen activators with low activity were not detected. Nevertheless, the resolving power of the method is sufficient for distinguishing between the properties of urokinase and of the other tissue activators.

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