

and animal plasminogen into plasmin (Fig. 1) as well as the hydrolysis of lysine and arginine esters; the esterolytic activity of the activator, in contrast to plasmin, is not inhibited by soybean trypsin-inhibitor. Its active site is not accessible to bigger molecules, as evidenced by a strong reduction of its proteolytic activity compared with plasmin. Fibrin, for example, is cleaved not at all by the SK-induced activator.

Plasmin also combines with SK to form an activator complex. It is suggested that the activator has an active site in common with plasmin, with the difference that it is partially sterically blocked by SK. This would explain that the esterolytic activity of the SK-induced activator equals the activity of plasmin, but that the esterolytic activity of the activator is not abolished by soybean trypsin-inhibitor and that large protein molecules are not cleaved in contrast to plasmin.

It remains still to be explained how the active site is generated from PP by SK. An autocatalytic process triggered by SK may be assumed.

Pharmacology of Thrombolysis: Urokinase

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Urokinase of human origin, the naturally occurring plasminogen activator found in human urine, possesses many of the biochemical properties required of an ideal thrombolytic agent.

First prepared on a pilot planned scale by Ploug and Kjeldegaard some 14 years ago, serious study of this agent in man only became possible in 1961 when two pharmaceutical companies successfully prepared material conforming to the following specifications: (1) The material was of high-specific activity, 35 000 CTA urokinase units/mg protein (crystalline urokinase has a specific activity approximately three-fold greater than the clinical grade material). (2) The preparation was essentially non-toxic in the experimental animal. (3) The preparation had been heat-treated at 56°C for 10 hours for the purpose of virus inactivation, and, (4) it passed stringent specifications for absence of thromboplastic components (components which contaminate the urine, from which it was prepared).

Studies in man have shown: (1) the material to produce highly-controllable and predictable states of enhanced plasma thrombolytic activity when administered on a dose/body weight basis; (2) have shown the material to be essentially non-toxic in man when administered in therapeutic doses; (3) human urokinase is non-antigenic to man; (4) human urokinase has an extremely high therapeutic

ratio; that is, it produces a maximal increase in plasma thrombolytic activity with only a mild induced blood coagulation defect; and, (5) as a consequence of (4), plasma-plasminogen levels are well maintained (approximately 30% of normal), even in states of greatly enhanced plasma thrombolytic activity, (6) Finally, urokinase possesses a most valuable property not found in any other thrombolytic agent; that of being a most flexible drug capable of inducing either a mild prolonged thrombolytic state with minimal disturbance to the blood coagulation system or a state of intense plasma thrombolytic activity for shorter periods of time.

Altogether, clinical experience with urokinase has shown this drug to approximate to that of the ideal thrombolytic agent. Unfortunately, urokinase is a most expensive drug, and, consequently, financial and administrative difficulties have greatly impeded developmental work at the clinical level. It should, however, be emphasized that the slow pace of development has been entirely due to administrative and financial difficulties and not due to any defect in the drug or its preparations.

A substantial possibility exists that urokinase may ultimately be produced on a commercial scale by tissue culture methods with a substantial reduction in cost.

Basic Biochemical and Pharmacological Properties of Brinase

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Brinase is a fibrinolytic and thrombolytic enzyme from the mould *Aspergillus oryzae*. Like plasmin it is not specific for fibrin only, but attacks proteinaceous substrates like casein, gelatin, and denatured haemoglobin. Brinase degrades fibrinogen roughly six times faster than it lyses fibrin, which compares favourably with trypsin, chymotrypsin, and the other proteolytic enzymes from *Aspergillus oryzae*, all having significantly higher fibrinogenolytic activity.

On fibrin plates brinase produces more marked lysis zones on heated than on unheated plates, suggesting that the enzyme is devoid of activator activity, which is verified using purified plasminogen. This implies that brinase does not consume the endogenous plasminogen, when used for thrombolysis.

The proteolytic enzyme inhibitors soybean trypsin inhibitor, ϵ aminocaproic acid, and Trasylol do not affect the enzymatic activity of brinase. The only efficient antidote at present known is plasma.

Electrophoresis shows that inhibitors towards brinase are localized in the α_1 and α_2 globulin

fractions. The α_2 macroglobulin fraction contains a reversible inhibitor and the α_1 fraction an irreversible inhibitor. The properties and electrophoretic mobilities of the brinase inhibitors appear similar to, if not identical with, the plasmin inhibitors in serum.

The inhibitor level in serum to a large extent determines the amount of brinase tolerated by an organism. Experiments in cats showed that significant changes in fibrinolytic activity on fibrin plates, fibrinogen level, whole blood clotting time, and blood pressure occurred only when the inhibitors were close to the point of exhaustion. However, in cats and rabbits experimentally induced venous thrombi are lysed by doses of brinase that do not exhaust the inhibitor capacity of the animal and do not cause apparent signs of toxicity (Bergkvist and Svård, 1964).

The *in vitro* addition of small doses of brinase to cat plasma shortens clotting time. Further increase

of the concentration of brinase induces slight hypocoagulability, only occurring when the inhibitor capacity is surpassed. The importance of the inhibitors for the effects of brinase on coagulation was also demonstrable *in vivo*. The administration of brinase in a total dose of 3 mg/kg to a cat predicted to tolerate 5-6 mg/kg caused only minor changes in coagulation parameters. Continuous infusion of 5 mg/kg of brinase induced moderate changes only which returned to pretreatment values within three hours.

When added *in vitro* to rat, rabbit, or human platelet-rich plasma brinase diminishes the tendency of platelets to adhere to glass and to each other as demonstrated by several *in vitro* techniques (Bygdeman, 1967; Svård, 1966) *In vivo* experiments on rabbits and rats showed that the reduced platelet aggregability induced by brinase decreased the tendency to develop thrombosis.