Fibrinolytic Proteases in Snake Venoms

Some of the snake venoms, mainly those from pit-vipers, have a relatively potent proteolytic activity. It could be proved that the venom-proteases differ from other proteolytic enzymes in the substrate specifity and inactivation by inhibitors[1,2]. Less attention has been paid to the influence on the fibrinolytic system in the blood.

Out of 24 various venoms, 2 with the strongest fibrinolytic and proteolytic activity were selected: *Agkistrodon piscivorus* and *A. contortrix* and compared with plasmin and trypsin.

If the bovin fibrinogen solution was incubed with venoms it gradually lost its ability to be converted into fibrin by thrombin, i.e. it was either denatured or split-off. There was no significant difference in the course of this action if plasminogen was incubated for 10 min at 37 °C with toxin or if toxin alone was added to the fibrinogen solution. (Figure 1). Hence it may be concluded that the venoms have practically no plasminogen-activating properties.

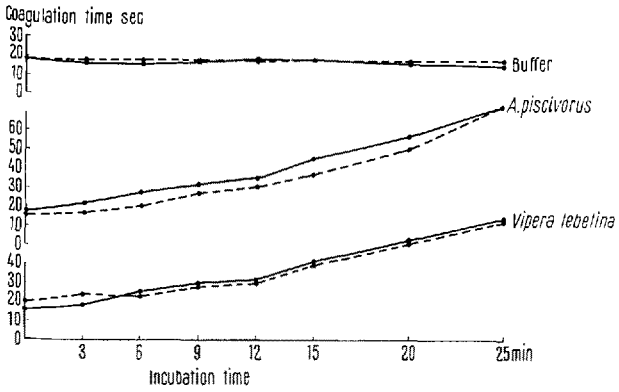

Fig. 1. Fibrinogenolysis by toxins and plasminogen. ----- toxin (buffer) + buffer; —— toxin (buffer) + plasminogen. Fibrinogen 1%, toxin 0.1%, plasminogen 0.2%.

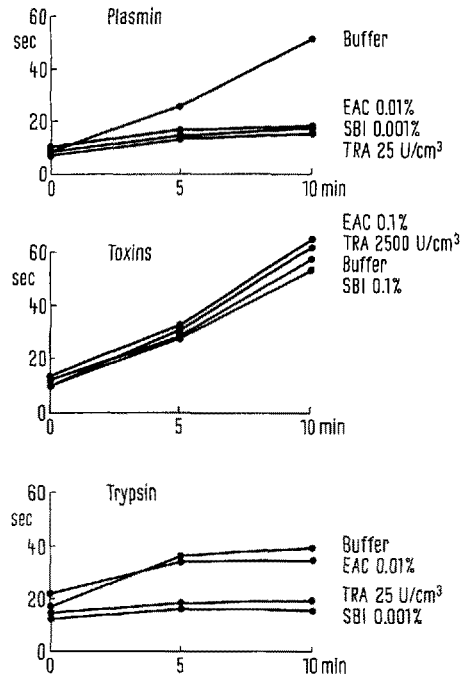

Fig. 2. Quantitative relations between fibrinogenolytic enzymes and their inhibitors. Incubation mixture: 0.5 cm³ fibrinogen (1%), 0.5 cm³ enzymes, 0.25 cm³ inhibitors. Enzymes and inhibitors preincubated for 20 min. Abscissa: clotting time, ordinata: incubation time.

The fibrinolytic activity of 300 U/cm³ of plasmin (0.2%) can be compared in the case of *A. piscivorus* venom with 0.025% and in the case of *A. contortrix* venom with 0.05% solution. In compliance with these quantitative relations, various inhibitors in different concentrations were added to the tested system. It was found that plasmin was inhibited by 0.01% E-aminocaproic acid (EAC), 0.001% soya-bean inhibitor (SBI) and 25 U/cm³ Trasylol (TRA); the same applies, with the exception of EAC, for trypsin. Even a 100 times stronger concentration of these inhibitors had hardly any effect upon the fibrinolytic activity of venoms (Figure 2).

GRUEDLINGERS method[3] – clot formation and lysis assessed by plotting changes of turbidity of the tested system against time – was used to ascertain the fibrinolytic properties of the venom-plasmin mixtures and the blocking action of the various inhibitors of the proteolytic enzymes. It was found that the venoms act synergically with plasmin and are able to paralyse to some extent the inhibitory effect of EAC, SBI, TRA (Figure 3).

From fibrinogen molecule 2 polypeptides (D and E) with antithrombin and antipolymerase activity (antithrombin VI) are split-off by plasmin. The inhibitory

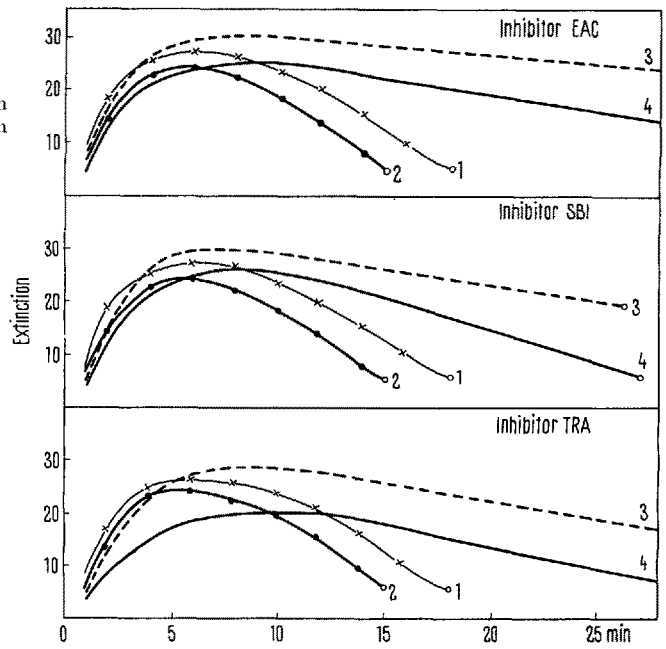

Fig. 3. Synergic action of toxins with plasmin and their influence on plasmin inhibitors. Constant mixtures: 1 cm³ fibrinogen (1%), 1 cm³ plasmin (500 U/cm³), 0.2 cm³ thrombin (10 NIH/cm³). Added: (1) 1 cm³ buffer, (2) 0.5 cm³ toxin + 0.5 cm³ buffer, (3) 0.5 cm³ inhibitor + 0.5 cm³ buffer, (4) 0.5 cm³ toxin + 0.5 cm³ inhibitor.

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effect on fibrinogen-conversion of the split products produced by plasmin and toxins was tested, and it could be proved that snake venoms elicit a greater activity of antithrombin VI within their split products than does plasmin.

In experiments in vivo, sublethal doses of *A. piscivorus* venom (400 μg/100 g) were injected s.c. to white rats. A routine blood coagulation check-up was performed in the time intervals of 30 min, 2 h and 24 h respectively. Except for a slight hypercoagulability of the whole blood in the first 30 min, no significant changes were observed, nor was the fibrinogen content decreased in plasma of experimental animals. This was explained by the fact that snake venom fibrinolysins are inhibited by whole serum (human, bovine, dog, guinea-pig, rabbit and rat).

The lytic activity of plasmin is controlled in the blood through antiplasmins derived from α_1 and α_2 globulin fractions[4,5]. Through the courtesy of Prof. HABERMANN, Pharmakologisches Institut, Giessen and Behringwerke, Marburg-Lahn, we obtained antiplasmin α_1 and macroglobulin α_2 and were able to compare their inhibitory effect on plasmin, trypsin and toxins (Figure 4). Antiplasmin α_1 inhibited fibrinogenolytic and caseinolytic activity of plasmin and trypsin completely, α_2 was effective against plasmin only. None of the isolated plasma antiplasmins effected any of the lytic activities of venoms.

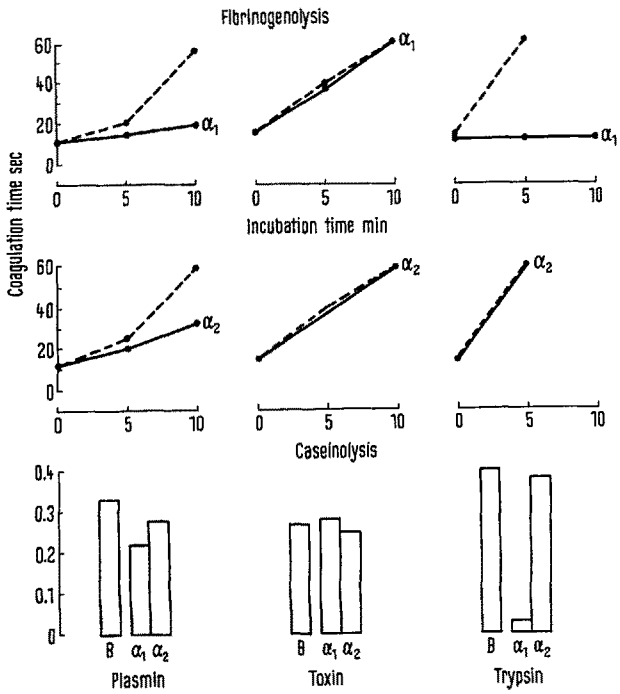

Fig. 4. Inhibition of the fibrogenolytic and caseinolytic properties of plasmin, trypsin and toxin by α_1 and α_2 antiplasmins.

Zusammenfassung. Fibrinolysine zweier Schlangengifte haben — im Gegensatz zu Plasmin und Trypsin — keinen Plasminogen-aktivierenden Effekt, noch sind sie durch Proteasen-Inhibitoren gehemmt. Sowohl in vivo wie auch in vitro sind sie durch Plasmaproteine inaktiviert, was jedoch nicht auf α_1 und α_2-Antiplasmine zurückzuführen ist. Die Spaltprodukte der Toxin-Fibrinogenolyse besitzen einen hohen Gehalt an Antithrombin VI.

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The Citrate Cleavage Enzyme Activity in Chick Embryo and Chicken Liver during Development

The cleavage of citrate might be regarded as a pathway for the supply of both acetyl CoA and oxaloacetate to the cytoplasm (SRERE[1,2]). The concentration of citrate cleavage enzyme (E.C. 4.1.3.6.) varies in accordance with its presumed role as a major source of acetyl units in lipid biosynthesis. While its level is depressed in the livers of diabetic and starved rats, it increases either after refeeding the animals with a rich carbohydrate diet (KORNACKER and LOWENSTEIN[3,4]) or after administration of insulin (SHRAGO and LARDY[5]). Moreover, a large increase in the activity of the citrate cleavage enzyme occurs in the rat mammary gland from the onset of lactation up to weaning (SPENCER and LOWENSTEIN[6]). In suckling rats the citrate cleavage enzyme decreases after birth from higher prenatal levels in both liver and adipose tissue, and increases slowly with weaning reaching adult levels (HAHN and DRAHOTA[7]).

No extensive investigation has been done on the relationship between the level of citrate cleavage enzyme and the carbohydrate metabolism, so that it is still doubtful if the enzyme plays a role in gluconeogenesis. The amount of phosphoenolpyruvate synthesized from citrate under normal conditions is quite significant for the rate of gluconeogenesis, even though the citrate is a poor substrate for phosphoenolpyruvate formation in rat liver if compared with other metabolites such as aspartate, fumarate or malate (SHRAGO and LARDY[5]).

To elucidate the role of citrate cleavage enzyme in the gluconeogenesis, its level has been measured in high speed supernatants of liver of both chick embryo and chicken. The results are shown in the Figure.

Material and Methods. In the liver of chick embryo, the gluconeogenesis rate is high, reaching a maximum on the sixteenth day of incubation and falling towards hatching

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