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Activation of Fibrinolysis by a Lymphogenic Activator in the Rat

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Summary

The plasminogen concentration in rat lymph was found to be on average 40% of the plasma concentration. The antiplasmin level was only 27% of the plasma level. On heated fibrin plates a distinct fibrinolysis in the lymph, collected over a period of 7 days, was found. The simultaneous lack of enzymatic activity in the plasma suggested the presence of a lymphogenic activator. Further evidence for the importance of the lymphogenic activator in blood fibrinolysis was found: plasminogen determinations performed in rat plasma during a period of 7 days of continuous lymph flow revealed significantly higher values compared to a control group. The euglobulin lysis time in the plasma of cannulated animals was significantly prolonged. The plasminogen consumption in the control animals was interpreted as being due to a continuous stress-induced activation of blood fibrinolysis by the lymphogenic activator.

The presence of coagulation factors in the lymph of experimental animals and humans is well established (1) but rather little is known about the fibrinolytic system of the lymph. Although a high content of plasminogen activator in human lymph nodes has been reported (2) no data are available concerning its significance in lymph and plasma fibrinolysis. The present communication deals with determinations of some components of the fibrinolytic system in rat lymph and rat plasma during the course of a long-term cannulation of the thoracic duct.

Material and Methods

The investigations were carried out in female SIV-50 rats, weighing 200-250 g. After thiobarbital anaesthesia the abdominal part of the thoracic duct was cannulated with a polyethylene cannula according to a technique described by *Bollman* (3). For 7 days the animals were kept in semirestraining cages. Lymph samples were collected in glass tubes containing 50 units/ml heparin to prevent clotting. The sampling of each lymph specimen lasted from 60 to 120 minutes. Blood samples were obtained by aortic puncture.

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Plasminogen was determined according to the modified caseinolytic method of *Alkjaersig* et al. (4). the activation of the lymph and plasma samples was carried out with human urokinase (Green Cross, Osaka). The antiplasmin levels, measuring the immediate and the slow reacting inhibitors, were estimated by the proteolysis inhibiting capacity of the lymph and plasma samples against porcine plasmin (*Novo, Copenhagen*) in a caseinolytic assay. Plasmin activity was detected by the heated fibrin plate method according to *Astrup and Müllertz* (5). If plasmin activity was present on the plates the result was designated as positive; a quantitation, measuring the amount of activity, was not performed. The euglobulin lysis time was carried out by acidification of the plasma to pH 5,2 (6).

The thoracic duct was cannulated in 30 animals. 30 animals which were only subjected to a sham operation but otherwise treated in the same way served as control group. One, four and seven days after continuous lymph flow the plasminogen and antiplasmin levels as well as the plasmin activity in lymph and plasma of 10 cannulated animals and in the plasma of 10 control animals were determined. Daily lymph specimens were assayed for plasmin activity. Seven days after the thoracic duct cannulation and the sham operation the euglobulin lysis time was performed.

Results

Table 1. Plasminogen and antiplasmin level in lymph and plasma of rats (n = 30).

	plasminogen U/ml	S.D.	antiplasmin U/ml	S.D.
lymph	77	17	21	6
plasma	193	31	79	15

Table 1 shows the plasminogen and antiplasmin concentrations in lymph and plasma of 30 animals. On average the plasminogen concentration of the lymph is 40% of the plasma concentration, whereas the antiplasmin level is 27% of the plasma level.

Figure 1 shows a high percentage of positive plasmin activity in the lymph on the operation day, whereas on day 1 after the operation almost no activity is present. On the following days increasing numbers of animals show positive results in their lymph specimens and on day 7 plasmin activity is constantly found. During this entire 7 days period we found plasmin activity in the plasma only on the operation day. From day 1 to 7 all blood samples gave negative results on heated fibrin plates.

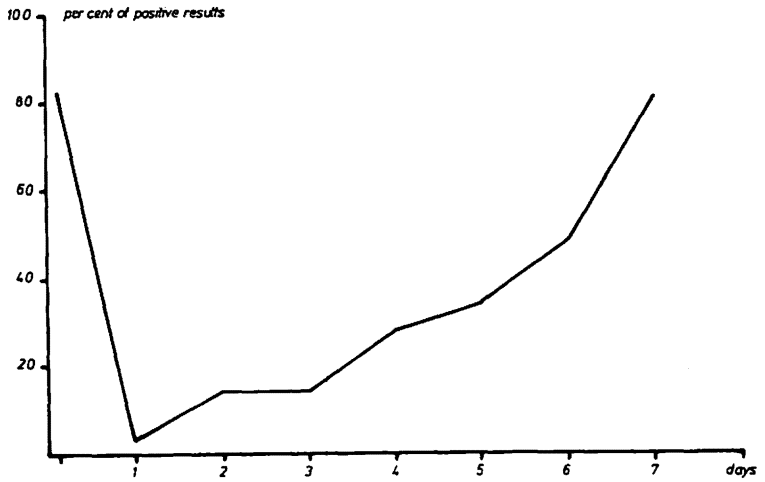

Fig. 1. Positive results of lymph samples on heated fibrin plates during 7 days of thoracic duct cannulation

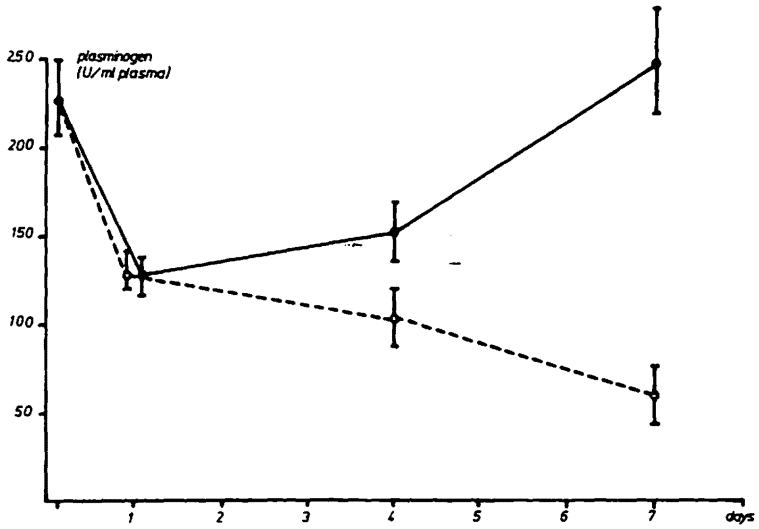

Fig. 2. Plasminogen level in the plasma of thoracic duct cannulated and sham-operated control animals (solid and dashed lines respectively)

Fig. 2 shows the plasma plasminogen content of thoracic duct cannulated animals in comparison to control animals. During the time of the experiment the antiplasmin levels of the plasma did not vary significantly in both groups. There is an initial decrease of the plasminogen level in the sham operated and the thoracic duct cannulated animals. In the control group the initial drop of the plasma plasminogen is followed by a further decrease. In the thoracic duct cannulated group the plasma plasminogen level increases again and reaches high normal values on day 7 after operation.

The euglobulin lysis time of the plasma of animals after 7 days of continuous lymph flow was 210 minutes (± 30 minutes) compared to 150 minutes (± 15 minutes) in the control group.

Discussion

Our results show that the ratio of plasminogen to antiplasmin is shifted significantly towards the plasmin precursor in rat lymph. The presence of a distinct fibrinolysis in the lymph, as shown by the fibrin plate method, and the simultaneous lack of fibrinolysis in the plasma may, however, only partly be explained by the lower inhibitor concentration in the lymph. The observation that even 7 days after the cannulation procedure there was no plasma fibrinolysis detectable, suggested the presence of a lymphogenic plasminogen activator. In constant experimental conditions the plasminogen level will be an index for an activation of the fibrinolytic system, a decrease of the levels indicating a consumption by plasminogen activation. It was demonstrated that the plasma plasminogen content in thoracic duct cannulated animals, after an initial decrease, rapidly increased to high normal values again. In the sham operated control group the plasminogen level decreased continously until the end of the experiment. The initial drop of the plasminogen level in both groups may be due to a stress induced activation by the operation (7). In the control group the further decrease may be interpreted too

by the continuous stress, caused by the confinement of the animals in the semi-restraining cages. Since the thoracic duct lymph was not allowed to recirculate into the systemic blood, the increase of the plasminogen level is possibly caused by the lack of an activation by a lymphogenic activator. Although the plasminogen level in the control group was obviously decreased the euglobulin lysis time in the plasma was significantly shorter than in the cannulated group. This fact further supports the thesis of the existence of a lymphogenic activator, since the euglobulin lysis time mainly measures activator activity. Recently *Szcepanski and Nilsson* (8) demonstrated that fibrinolytic activity during peripheral venous occlusion in rats was mainly related to the loose, connective tissue in the vicinity of the vessels. Since those fibrinolytic enzymes can only be removed by the lymph vessels, our present results again suggest that the lymphatic system participates in the regulation of blood fibrinolysis.

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