EFFECT OF TRYPSIN ON INTRAVASCULAR AGGREGATION OF ERYTHROCYTES

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There is evidence that proteolytic enzymes with wide substrate specificity (fibrinolysin, trypsin) can stimulate platelet aggregation [8, 10]. The importance of these findings is due to the fact that an increase in proteolytic activity in the blood is a fairly frequent phenomenon and can take place in many different pathological states (shock, acute cardiovascular failure, etc.) and also during thrombolytic therapy (with plasmin, streptase, and urokinase). In previous experiments in vitro the present writers showed that under the influence of proteolytic enzymes the elasticity of erythrocytes is reduced and their aggregation takes place [3, 4]. It has also been shown that acetylsalicylic acid inhibits the aggregating action of fibrinolysin and trypsin on erythrocytes.

The object of the present investigation was to study the effect of trypsin on intravascular aggregation of erythrocytes in experiments in vivo and the action of acetylsalicylic acid and pentoxyphyllin on this process.

EXPERIMENTAL METHOD

Experiments were carried out on 15 rats weighing 180-220 g. Aggregation of the erythrocytes in the mesenteric vessels was observed by means of an apparatus for intravital microscopy mounted on the base of the BMI-6 microscope. The animals were anesthetized with pentobarbital during the investigation. Trypsin (from the Medical Preparations Factory, Leningrad Meat Combine), in a dose of 40-50 mg/kg body weight, was injected into the caudal vein of the rat in the course of 2-3 sec. To prevent the development of hypercoagulation, the trypsin was given together with heparin (1000 units/kg body weight).

The animals were divided into three groups: Group 1 consisted of rats receiving trypsin with heparin, group 2 rats to which administration of these preparations was preceded by peroral administration of acetylsalicylic acid in a dose of 100 mg/kg, in the form of a 1% suspension in starch gel, and group 3 consisted of animals receiving pentoxyphyllin intravenously in a dose of 20 mg/kg before the injection of trypsin.

The state of the microcirculation was studied in the animals of all groups during the 30 min before and after administration of trypsin.

EXPERIMENTAL RESULTS

Before receiving trypsin, none of the animals showed any appreciable changes in the microcirculation.

Between 15 and 20 sec after administration of trypsin, aggregates of erythrocytes began to appear in the mesenteric vessels of the animals of group 1 and the velocity of the blood flow progressively diminished. After 3-4 min the blood flow was very considerably slowed, and in some vessels to-and-fro or retrograde movement of the blood was observed. On the whole, the changes in the microcirculation resembled the sludging syndrome (Fig. 1). These phenomena, without any subsequent significant changes, were observed during 1.5-2.5 h after injection of the proteolytic enzyme.

In the animals of group 2, which received acetylsalicylic acid 2 h before intravenous infusion of trypsin, a different picture was observed. Trypsin did not cause aggregation of the erythrocytes. Immediately after

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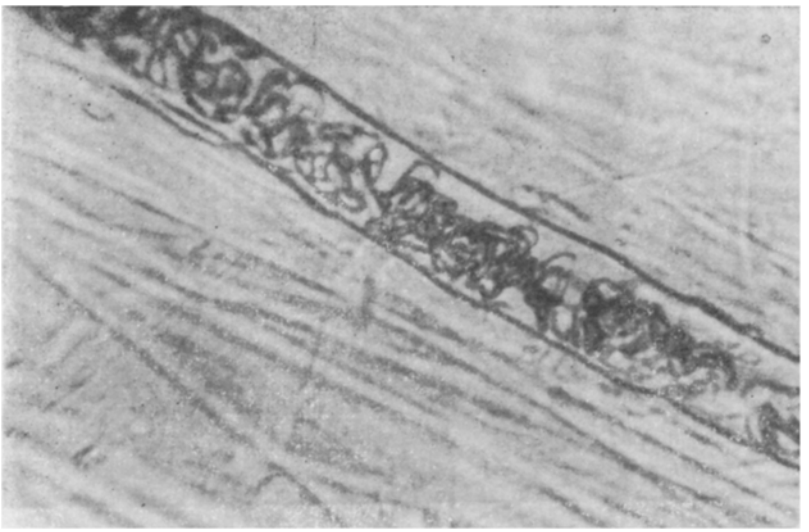

Fig. 1. Mesentery of small intestine of rat after injection of trypsin. 1020×.

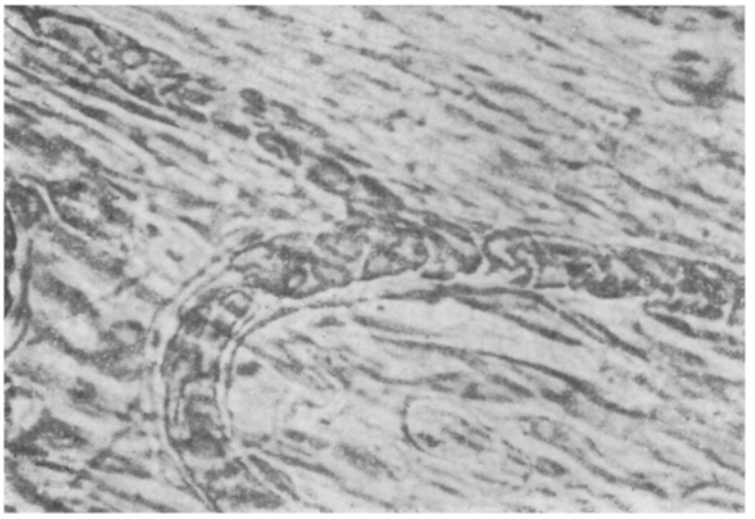

Fig. 2. Mesentery of small intestine of rat after injection of trypsin preceded by peroral administration of acetylsalicylic acid. 1020×.

its injection there was a very brief slowing of the blood flow, followed by instantaneous restoration of the circulation to its initial level (Fig. 2).

In the animals of group 3, which received pentoxyphyllin 2 h before the experiment, trypsin caused no microcirculatory changes. However, if the pentoxyphyllin was injected only 15-20 min before the experiment it did not prevent aggregation of erythrocytes induced by trypsin. The microcirculatory disturbances corresponded to the changes taking place in the animals of group 1.

The results are evidence that in vivo trypsin induces intravascular aggregation of erythrocytes. This phenomenon is not due to thrombin generation, for it develops in the presence of severe hypocoagulation associated with the use of heparin (after death of the animal the blood remained liquid for 1 h). The results do not agree with the earlier view that heparin inhibits the harmful action of trypsin on erythrocytes [6]. Heparin evidently can even aggravate the microcirculatory disturbances because of its aggregating action on erythrocytes [5] and platelets [7]. The results of this investigation also contradict observations showing that trypsin causes destruction of erythrocytes [6]. On the contrary, in the presence of marked aggregation of erythrocytes

stimulated by trypsin, hemolysis was not observed in experiments in vitro or on animals. Even a significant change in shape of the erythrocytes and their aggregation are thus not always accompanied by hemolysis. The mechanisms causing these processes are evidently not completely identical.

The writers postulated previously,on the basis of the results of experiments in vitro,that the aggregating action of trypsin is based on activation of phospholipase A by the enzyme. Lysoderivatives and free fatty acids formed by hydrolysis of phospholipids subsequently "trigger" aggregation of the blood cells [2, 3]. The most important evidence in support of this view is that given by the experiments with acetylsalicylic acid and pentoxyphyllin. Both agents completely prevented intravascular aggregation of erythrocytes induced by trypsin. The principal factor in the disaggregating action of acetylsalicylic acid is its prevention of hydrolysis of phospholipids in the blood cell membranes [4].

Pentoxyphyllin may evidently act in a similar way. As data in the literature show, it inhibits phosphodiesterase, thus leading to accumulation of cyclic AMP in the blood cells [11]. This blocks hydrolysis of phospholipids under the influence of aggregating agents, a process which has more recently been suggested as the explanation of the antiaggregating effect of cyclic AMP [9].

The present experiments thus indicate the very important role of increased proteolysis in the development of intravascular aggregation of erythrocytes. Correction of the microcirculatory disturbances under these conditions can be achieved by the use of substances stabilizing erythrocyte membranes and preventing hydrolysis of their phospholipid components.

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