The results thus point to the existence of close direct interconnection between LPO and PLH processes in nerve endings.

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EFFECT OF ANTICOAGULANTS ON HUMAN PLASMA TRYPSIN-LIKE PROTEINASE ACTIVITY

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UDC 615.273.53.015.4:[616.153.1:577.152.34].07

KEY WORDS: heparin; blood proteinases; anticoagulants

Changes in the proteolytic activity of human blood are nowadays an important parameter in clinical biochemistry in the diagnosis of several diseases, such as acute pancreatitis, shock of varied etiology, myocardial infarction, etc. [1, 2]. Reliable determination of proteolytic activity is therefore extremely important. Meanwhile anticoagulants (usually heparin, sodium citrate, or a combination of both) are widely used in clinical practice. The need for this is linked with the use of new methods of treatment, such as plasmapheresis, double and cascade ultrafiltration, and biospecific sorption on affinity sorbents [6, 7]. The aim of this investigation was to study the effect of anticoagulants on activity of human blood trypsin-like proteinases.

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 2, pp. 177-179, February, 1989. Original article submitted October 23, 1987.

0007-4888/89/0002-0186$12.50

TABLE 1. Dependence of TA of Blood Proteinases on Anticoagulant Used

No. of donor	TA, mU/ml				
		plasma			
	serum	citrate (0.38%)	heparin (5 U/ml) + citrate, (0.38%)	heparin (5 U/ml)	heparin (100 U/ml)
1	34±2	42±2	490±56	491±91	997±10
2	34±6	38±2	177±40	289±32	1142±16
3	32±2	32±4	180±20	338±16	619±8
4	40±3	24±1	193±16	289±32	852±16
5	44±2	26±2	156±12	297±40	668±57
6	21±1	17±1	161±40	217±40	651±8
7	17±3	12±1	201±24	249±8	704±60
8	36±4	30±6	156±16	349±69	675±16
Mean	32±3	26±2	211±28	325±32	785±24

EXPERIMENTAL METHOD

Heparin ("Sigma," USA and "Gedeon Richter," Hungary), sodium citrate ("Reakhim," USSR), N-α-benzoyl-L-arginine-p-nitroanilide and bovine trypsin ("Sigma") were used.

Blood was obtained from healthy donors (women aged 21 to 37 years) and heparin (Gedeon Richter) and (or) sodium citrate was added to it (in a volume of 0.1 of the volume of blood). Blood cells were removed by centrifugation (1500g, 30 min, 14°C). The plasma thus obtained was kept at 4°C in the presence of 0.02% sodium azide for not more than 3 days. To obtain serum, the donated blood was incubated for 1 h at room temperature, after which the clot was removed by centrifugation under the same conditions.

Trypsin-like activity (TA) was determined by the method in [3]. The substrate was a 0.1M solution of N-α-benzoyl-L-arginine-p-nitroanilide in dimethyl sulfoxide. To 2 ml of 50 mM Tris-HCl, pH 7.8, containing 20 mM $CaCl_2$, 20 μliters of a solution of the substrate was added, followed by 20-50 μliters of the test sample, and the kinetics of the change in optical density at 405 nm was recorded on a spectrophotometer. Enzyme activity was expressed in internal units, taking 1 unit as the amount of enzyme whose action leads to a change of optical density of 0.00664/min (in a 1-cm cuvette, under the conditions given above) [4].

To determine activity of trypsin inhibitors in the plasma or serum, 400 μliters of trypsin solution (0.1 mg/ml in 2.5 mM HCl, containing 20 mM $CaCl_2$) was treated with different quantities of the test sample, followed by adjustment of the volume of the sample to 800 μliters with 50 mM Tris-HCl, pH 7.8, containing 20 mM $CaCl_2$. The pH of the reaction mixture remained unchanged under these circumstances at 7.8. Samples were incubated for 20 min at room temperature, after which their enzymic activity was determined. From the curve of activity as a function of quantity of the specimen in the sample, the minimal volume of plasma or serum causing complete inhibition of trypsin activity was determined.

On determination of the dependence of TA of the serum on the heparin concentration in it, 200 μliters of serum was treated with heparin solution ("Sigma") and the volume of the sample was adjusted with physiological saline to 250 μliters. Next, after incubation for 20 min, enzymic activity was determined in the samples.

EXPERIMENTAL RESULTS

The results given in Table 1 show that heparin, added to blood in a concentration of 5 U/ml, caused a marked increase (of virtually an order of magnitude) in TA compared with serum, and a further increase in its concentration to 100 U/ml led to a further increase in TA. Meanwhile sodium citrate had no marked effect on TA. The use of a combination of citrate and heparin reduced the activating effect of heparin partially. Control experiments showed that the anticoagulants used did not contain impurities with TA. It can be seen from the results in Fig. 1 that dependence of TA on heparin concentration is described by a curve with saturation, and that maximal enzymic activity was reached at 800 U heparin in 1 ml of serum. A subsequent increase in the quantity of heparin in the serum did not lead to any increase in enzyme activity.

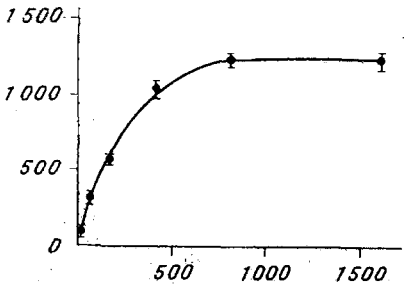

Fig. 1

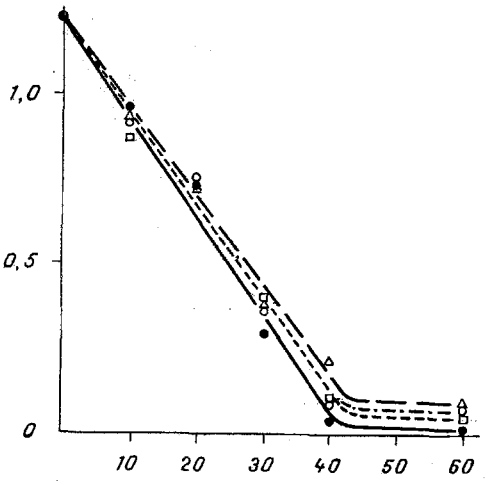

Fig. 2

Fig. 1. Dependence of serum TA on its heparin concentration. Abscissa, heparin concentration in sample (in U/ml); ordinate, TA (in mU/ml).

Fig. 2. Antitryptic activity of serum for donor 1 (Table 1) and plasma with various anticoagulants. Filled circles – serum, triangles – heparin, 5 U/ml; empty circles – heparin, 100 U/ml; squares – citrate, 0.38%. Abscissa, volume of plasma (in μliters); ordinate, trypsin activity (in conventional units).

We know that a polyanionic environment has an activating effect on some proteolytic enzymes [5]. The possibility cannot be ruled out that heparin, which is a polymer and carries a considerable negative charge, has a similar action on the blood trypsin-like proteinases.

Another possible explanation of the observed effect of activation of TA in the presence of heparin may be its action on the system of proteinases and their inhibitors, resulting in activation of the inhibitors and a corresponding rise of TA. To test this hypothesis we studied the antitryptic activity of plasma and serum trypsin inhibitors. Specimens of plasma and serum from donor 1 (Table 1) were used. The experimental results, given in Fig. 2, show that values of antitryptic activity of the serum and plasma, containing different anticoagulants, did not differ significantly. Thus activity of trypsin inhibitors was virtually independent of the type and concentration of the anticoagulants used. However, the possibility cannot be ruled out that heparin, as a polyanion, may bind inhibitors of other proteinases in plasma, thereby shifting equilibrium of the reaction of enzyme–inhibitor complex formation toward its dissociation. As a result, proteinases capable of degrading the synthetic substrate used may be released, thereby inducing an increase in TA.

It can be concluded from these results that total plasma TA from human blood depends on the type and concentration of the anticoagulant used.

When activity of proteinases (especially in patients before and after procedures connected with an extracorporeal circulation) is determined, control experiments must invariably be carried out and corrections made to allow for the effect of anticoagulants.

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