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THE ACTION OF ANTITHROMBIN III ON PLASMIN AND ACTIVATORS OF PLASMINOGEN

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Summary

1. Antithrombin III progressively inhibited plasmin activity measured by hydrolysis of casein, fibrin, and synthetic substrates. The presence of heparin potentiated the inhibitory effect of antithrombin III on plasmin.

2. Tissue and plasma activators of plasminogen and urokinase were not inhibited by antithrombin III.

Introduction

Agents are present in circulating blood, most tissues and many body secretions, including urine, which are capable of converting plasminogen to the active serine protease, plasmin (EC 3.4.21.7); the plasminogen activators from these various sources have not, however, been fully differentiated. Plasma also contains a number of protease inhibitors and of these alpha-2-macroglobulin [1] alpha-1-antitrypsin [2] Cl inactivator [3] and antithrombin III [4,5] have been shown to possess antiplasmin activity. The relationship of antithrombin III to activators of plasminogen is not defined.

Materials and Methods

Immunoelectrophoresis, quantitative radial immunodiffusion, double diffusion in agar gels and analytical acrylamide gel electrophoresis were performed as previously described [6].

Tissue activator was prepared from human myocardium by the method of Bachmann et al. [7] and urokinase was purchased from Leo Laboratories, Copenhagen, Denmark.

Plasma activator was prepared in a crude form from blood obtained from the arm veins of volunteers, after stimulation of activator release by venous occlusion, by gel filtration of separated plasma on Sephadex G-200 at 4° C. The

activator preparations were free of plasminogen, alpha-2-macroglobulin, alpha-1-antitrypsin and antithrombin III when examined by double diffusion in agar gels against antisera specific for these proteins.

Antithrombin III was prepared from cryoprecipitate-depleted human plasma by modifications of the methods of Abilgaard [8] and Rosenberg and Damus [9]. On analytical acrylamide gel electrophoresis and immunoelectrophoresis the preparations contained three proteins identified as antithrombin III, alpha-2 HS glycoprotein and alpha-1-acid glycoprotein.

The influence of antithrombin III on the fibrinolytic properties of plasmin, urokinase, tissue activator and blood activator was assessed using unheated fibrin plates. Antithrombin III and enzyme were incubated together at room temperature before 30 μl aliquots were applied to the fibrin plate. The area of lysis was assessed after 24 h incubation at 37° C.

Antiplasmin activity was measured, in addition, using casein and the synthetic materials acetyl-lysine methyl ester (Cyclo Chemical Corp., Los Angeles, U.S.A.) and benzoyl-1-phenylalanyl-1-valyl *p*-nitroanilide (AB Bofors, Nobel Division, Peptide Research, Molndal, Sweden) as substrates. Inhibition of plasmin (lyophilised, AB Kabi, Stockholm, Sweden) in these systems was assessed by incubation of plasmin with antithrombin III prior to addition of substrate. Residual plasmin was determined by minor modifications of methods previously described [6,10].

Results

Results of typical experiments are presented. Each has been repeated three or four times with qualitatively similar results.

Inhibition of plasmin by antithrombin III

The proteolytic, esterolytic and fibrinolytic activities of plasmin were inhibited by antithrombin III: the extent of plasmin inhibition was related to the concentration of antithrombin III in the initial incubation mixture (Table I).

Using S-2160 and casein as plasmin substrates it was established that the inhibition of plasmin by antithrombin III was progressive with time. The addition of heparin to the initial incubation mixture of plasmin and antithrombin III resulted in more rapid neutralisation of plasmin activity measured using casein and S-2160 as substrates.

Antithrombin III and activators of plasminogen

The concentrations of tissue activator, blood activator and urokinase were adjusted such that the areas of lysis produced by the uninhibited activator were approximately equal. In the presence of antithrombin III, final concentration 10 mg per 100 ml, areas of lysis of 72, 128, 89 mm^2 were produced by blood activator, tissue activator and urokinase and in the absence of antithrombin III areas of 76, 132, 88, respectively. Each value is the mean of four determinations. It is concluded that antithrombin III does not influence the activity of physiological plasminogen activators.

TABLE I

INHIBITION OF PLASMIN BY ANTITHROMBIN III, MEASURED USING CASEIN, FIBRIN, Ac-Lys-Me ESTER AND S-2160 AS SUBSTRATES

Antithrombin III concentrations are those in the initial incubation mixtures (mg per 100 ml). The concentrations of plasmin used were 0.125 mg per ml (caseinolysis), 0.2 mg per ml (esterolysis), 0.5 mg per ml (fibrin plate)-1.25 mg per ml (S-2160), all concentrations referring to the initial incubation mixture.

Caseinolysis		Esterolysis		Fibrinolysis		S-2160	
Concentration of antithrombin III	Residual activity (μg acid-soluble tyrosine-like material released)	Concentration of antithrombin III	Residual activity (μmol ester hydrolysed)	Concentration of antithrombin III	Residual activity (mm^2)	Concentration of antithrombin III	Residual activity (% control)
22.5	8	24	0.02	12.5	115	15	52
18	11	18	0.14	9.4	141	11.2	58
13.5	14.5	12	0.22	6.3	181	7.5	68
9	20	6	0.40	3.1	221	3.8	87
0	34	0	0.63	0	294	0	100

Discussion

Antithrombin III has been noted to inhibit a number of serine proteases including trypsin, thrombin and plasmin: the present study confirms the progressive antiplasmin properties of physiological concentrations of antithrombin III and the potentiating action of heparin on this property [5]. In its progressive nature antithrombin III resembles alpha-1-antitrypsin [6], but differs from alpha-2-macroglobulin, the other major protease inhibitor of human plasma [1].

In contrast, urokinase, plasma activator and tissue activator were not inhibited by antithrombin III. Urokinase and tissue activator from porcine heart and parotid glands have, based on their inactivation by treatment with diisopropylphosphorofluoridate, an active site requiring the participation of serine residues [11—13]. They therefore resemble the serine protease chymotrypsin which is also not inhibited by antithrombin III [4].

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