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Anticoagulative effect of pepsin*

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Anticoagulative effect of pepsin is observed *in vitro* when its concentration is 36 μM and higher. This effect is due to inhibition of fibrin monomer polymerization. Protamine abolishes anticoagulative effect of pepsin. Pepsin does not influence platelet aggregation induced by ADP and collagen.

Anticoagulative effect of heparin and other glycosaminoglycans is connected with their polyanion character [1, 2]. Hence it seemed probable that pepsin with the isoelectric point at pH 1.0 may also demonstrate such an effect [3].

The aim of this report was to show the influence of pepsin on prothrombin, recalcification, celite-cephalin and thrombin times and on the amidolytic activity of thrombin. Also the influence of pepsin on platelet aggregation induced by adenosine 5′-diphosphate and collagen was examined.

REAGENTS AND METHODS

Adenosine 5′-diphosphate (ADP), fibrinogen, protamine, thrombin, Sigma (St. Louis, U.S.A.); collagen, Hormonchemie (Munich, Germany); pepsin A, Serva (Heidelberg, Germany); thromboplastin (thromborel S), Behringewerke A.G. (Marburg, Germany); H-D-Phe-Pip-Arg-pNA, Kabi Vitrum (Sweden); cephalite, bioMerieux (Marcy-l'Etoile, France).

Effect of pepsin on prothrombin, recalcification and celite-cephalin times was investigated in the system containing: 0.18 ml of human citrated plasma and 0.02 ml of pepsin (0.09 to 2.88 mM) or 0.02 ml NaCl (0.15 M) in control. To measure prothrombin time 0.2 ml of thromboplastin was added. Recalcification time was measured after addition of 0.2 ml of $CaCl_2$ (0.025 M). Celite-cephalin time was investigated after addition of 0.1 ml of cephalite and 0.1 ml of $CaCl_2$ (0.05 M). All measurements of clotting time were made at 37°C.

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Effect of pepsin on thrombin time was examined in the system containing: 0.18 ml of fibrinogen (0.25%) and 0.02 ml of pepsin (0.09 to 2.88 mM) or 0.02 ml NaCl (0.15 M) in control. After adding 0.2 ml of thrombin (8 U/ml) clotting time was measured at 37°C.

The effect of pepsin on the amidolytic activity of thrombin was investigated in a system [4] containing: 0.65 ml Tris/HCl buffer (0.1 mM, pH 8.4), 0.05 ml of pepsin (0.09 to 2.88 mM) or 0.05 ml NaCl (0.15 M) in control, 0.2 ml of H-D-Phe-Pip-Arg-pNA (0.75 mM) and 0.1 ml of thrombin (5 U/ml). Samples were incubated for 10 min at 37°C. The reaction was stopped by adding 0.1 ml 40% acetic acid and the absorbance was measured at 405 nm. The amount of released pNA was read from a calibrated graph prepared with the use of a standard solutions of this compound.

Neutralization of the anticoagulative effect of pepsin by protamine was investigated in the systems containing 0.18 ml of fibrinogen (0.25%), 0.02 ml of pepsin (0.36 mM), 0.2 ml of thrombin (8 U/ml) and alternatively 0.02 ml of protamine (0.18, 0.36, 0.72 mM) or 0.02 ml NaCl (0.15 M). Clotting time was measured at 37°C.

The influence of pepsin on blood platelet aggregation was investigated in the systems [5] containing: a) 0.9 ml of porcine platelet rich plasma, 0.1 ml of pepsin (0.36 mM) and alternatively 0.1 ml ADP (100 mM) or 0.1 ml of collagen (100 μg/ml) and transmitance was measured; b) 0.9 ml of porcine platelet rich plasma, 0.05 ml of pepsin (0.72 mM) and alternatively 0.05 ml of ADP (200 mM) or 0.05 ml collagen (200 μg/ml) and transmitance was measured.

RESULTS

Pepsin prolongs prothrombin, recalcification and celite-cephalin times in a concentration-dependent manner (Table 1).

Table 1. Influence of pepsin on prothrombin time, recalcification time and celite-cephalin time

Pepsin μM	Prothrombin time (s)	Recalcification time (s)	Celite-cephalin time (s)
0.0	15.3	210.7	75.0
4.5	18.3	210.3	75.3
9.0	22.0	215.0	75.0
18.0	28.3	230.7	75.7
36.0	36.0	248.3	84.3
72.0	42.3	312.7	92.7
144.0	50.0	365.0	162.0

Also pepsin prolongs thrombin clotting time (Table 2). However, it does not inhibit the amidolytic activity of thrombin against H-D-Phe-Pip-Arg-pNA.

Protamine at 18 μM concentration decreases and at 36 μM completely abolishes the prolongation of thrombin time by 0.36 mM pepsin (Table 3).

Table 2. Influence of pepsin on thrombin time and amidolytic activity of thrombin

Pepsin μM	Thrombin time (s)	pNA μM
0.0	16.3	108.3
4.5	16.7	108.3
9.0	16.7	108.3
18.0	16.7	107.7
36.0	26.3	108.3
72.0	34.0	109.0
144.0	41.3	108.7

Table 3. Neutralization of anticoagulative effect of pepsin by protamine

Test components	Protamine μM			
	0.0	9.0	18.0	36.0
	Clotting time (s)			
Protamin + NaCl	15.0	15.3	15.7	15.7
Pepsin* + protamine	26.7	26.3	16.7	15.3

* Pepsin concentration was 36 μM

Pepsin does not induce blood platelet aggregation and does not inhibit platelet aggregation induced by ADP and collagen.

DISCUSSION

The presented data show that pepsin prolongs prothrombin, recalcification, celite-cephalin and thrombin times. In these tests the generated or added thrombin induces the formation of fibrin monomers, their polymerization and formation of fibrin clot. The lack of inhibition of the amidolytic activity of thrombin excludes the effect of pepsin. It allows to suppose that the anticoagulative effect of pepsin depends on the formation of complexes with fibrin monomers thus making their polymerization difficult. The abolishment of the anticoagulative effect of pepsin by protamine with which it forms stable complexes supports this supposition [6]. It is also confirmed by the inhibition of polymerization of isolated fibrin monomers by pepsin (unpublished). Polymerization of fibrin monomers is also inhibited by fibrinogen degradation products [7], haemoglobin [8] and some synthetic polyamino acids [9].

An impairment of haemostasis manifested by persistent bleedings and haemorrhages difficult to control is observed in some pathologic conditions affecting gastric circulation [10, 11]. This pathology may result from the anticoagulative effect of hydrochloric acid [11, 12], the increased fibrinolytic activity of gastric blood vessels [13, 14], and perhaps the anticoagulative effect of pepsin as well. About 1% of pepsin synthesized in the principal cells of gastric mucosa gets into circulating blood where its concentration in general circulation is from 1.5 to 5.0 nM [15]. Local concentration of pepsin in gastric circulation is probably much higher. Blood flowing from blood vessels into stomach lumen will get into contact with pepsin of the gastric juice. Concentration of pepsin in the gastric juice may surpass 100 μM [16].

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