globin content in cells from the carotid artery, and their greater diameter. This separation is evidently of great physiological importance: young forms of red cells can exchange their oxygen for carbon dioxide more efficiently, for affinity of red cells for oxygen increases with age [5].

However, the cause of this effect is not clear. It may be that a definite role is played in this situation by hemodynamic differences at the point of branching of the vessels from the aortic arch.

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EFFECT OF HEPARIN ON ACTIVATION OF THE ANTICLOTTING SYSTEM BY α-THROMBIN

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α-Thrombin is a bioregulator of hemostasis and of the liquid state of the blood and is responsible both for blood clotting and for activating the anticlotting potential of the body [2]. The bioregulatory functions of α-thrombin are effected through a special site of its molecule — the recognition center for high-molecular-weight compounds, located outside the active center proper [2, 5]. For certain functions of α-thrombin to be realized, contact between the recognition center and the complementary site on the cell receptor is sufficient [2, 10]. For instance, the hormone-like activity of thrombin is manifested as excitation of the anticlotting system [2], and also as stimulation of chemotaxis of monocytes, activation of neutrophils, and aggregation of lymphocytes [9, 10]. The recognition center also makes an important contribution to stimulation of synthesis and release of prostacycline, to induction of aggregation of platelets and enhancement of their reactivity, to activation of the blood clotting inhibitor protein C, and other functions, by maintaining the unique specificity of α-thrombin for protein substrates and cell receptors [2, 3, 10]. The recognition site for high-molecular-weight compounds consists of a number of subcenters: the chemotaxic domain, the fibrinogen recognition site, and the cationic subcenter [10]. The latter is responsible for interaction of thrombin with heparin [10]. It has been postulated that the

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recognition center determines the direction of the action of α-thrombin in physiological reactions [5]. The functional activity of the enzyme can be modulated by selective blocking of the subcenters of the recognition center. For instance, binding of the cationic subcenter of α-thrombin by heparin activates the reaction with antithrombin III and partly blocks the clotting activity of the enzyme and its ability to activate platelets [2].

The aim of this investigation was to study whether the ability of α-thrombin to interact with specific receptors of the vascular bed, responsible for exciting the anticlotting system, can be regulated by heparin.

The writers showed previously [6] that α-thrombin, in the form of a complex with heparin, preserves 60% of clotting activity. We therefore used di-isopropylphosphoryl-α-thrombin (DIP-α-thrombin), which has lost its enzyme activity through blockade of the serine of its active center, but, like α-thrombin, still possesses its ability to activate the function of the anticlotting system [2], in these experiments. The state of the clotting and anticlotting systems was analyzed after perfusion of the carotid sinus zone of rabbits with DIP-α-thrombin alone, with a DIP-α-thrombin–heparin complex, or with heparin alone.

EXPERIMENTAL METHOD

Altogether 25 experiments were carried out on chinchilla rabbits: The humorally isolated carotid sinus zone of the rabbit, with its innervation intact, was perfused by the Heymans–Anichkov method. A constant rate of perfusion was maintained by means of a peristaltic pump. Continuously aerated Ringer–Locke solution in 0.4% glucose solution (pH 7.4, 37°C) was used as the nutrient solution. After dissection under local anesthesia with 0.05% procaine solution the carotid sinus zone was perfused with nutrient solution for 40 min, then with the test preparation in the same solution (volume 20 ml), after which it was again perfused with the solvent. Before perfusion of the test preparation and at certain time intervals thereafter blood samples were taken from the femoral vein, for determination of the thrombin time, total fibrinolytic activity, nonenzymic fibrinolysis [1], and enzymic fibrinolytic activity [8]. The results were subjected to statistical analysis. Perfusates of the test preparation were collected and analyzed for their heparin concentration by determining their ability to block the amidase activity of α-thrombin (against phenylalanyl-pipecolyl-arginine-p-nitroanilide) in the presence of antithrombin III [4] and of the formation of a complex with azure [13]. α-Thrombin with clotting activity of 1500 NIH units/mg protein, obtained as in [7] was used in the experiments; the DIP-α-thrombin was obtained by blocking the active center of α-thrombin with di-isopropyl fluorophosphate (DFP) in a final concentration of 1 mM. The reaction was conducted in Tris-phosphate buffer, pH 7.5, for 1 h at 20°C. The excess of DFP was removed by dialysis. The residual clotting activity of the DIP-α-thrombin did not exceed 0.1 NIH unit/mg of the preparation. The DIP-α-thrombin was used in a concentration of 2 μM. The DIP-α-thrombin–heparin complex was obtained with equimolar proportions of the components, using heparin from "Serva") with activity of 170 U/mg of the preparation. The purity of the equimolar complex was analyzed by gel-filtration on Sephadex S-200 [12]. In this concentration (2 μM) the complex was unable to produce lysis of unstabilized fibrin. Experiments with intravenous injection of heparin were carried out on 40 albino rats weighing 180-200 g. The test substances, in a volume of 1 ml, were injected into the jugular vein, from which blood samples were taken 5 min after injection of the drug, and the same parameters of the state of the anticlotting system were determined as in the case of perfusion of the carotid sinus zone of the rabbits.

EXPERIMENTAL RESULTS

In a series of experiments to study perfusion of the carotid sinus zone of rabbits with DIP-α-thrombin (1.8 μM) a normal response of the anticlotting system was observed, namely a statistically significant increase in thrombin time (by 22.3%), in enzymic fibrinolytic activity (by 52.4%), and in nonenzymic fibrinolysis (by 25.5%), reflecting activity of complexes of heparin, the humoral agent of the anticlotting system (Table 1). Heparin, secreted by the wall of the carotid artery, was found in the DIP-α-thrombin perfusates. The time course of secretion correlated with changes in the heparin level in the bloodstream. During the period of maximal secretion (the 3rd minute of the experiment) under 0.2 U heparin/ml was recorded by different methods, in agreement with the amount of heparin found in perfusates during activation of the anticlotting system with prothrombin I — the inactive precursor of α-thrombin [4].

In the experiments with control perfusion of the carotid sinus zone with albumin (2 μM) hypocoagulation in systemic circulation was not observed and no heparin was found in the albumin perfusates.

TABLE 1. Changes in Parameters of State of Anticlotting System (in % of control level) after Perfusion of Carotid Sinus Zone of Rabbits with DIP-α-Thrombin–Heparin Complex, or with Heparin or DIP-α-Thrombin alone (M ± m)

Substance used for perfusion	Time after perfusion, min	Total fibrinolytic activity	Nonenzymic fibrinolysis	Thrombin time	Enzymic fibrinolytic activity
2 μM DIP-α-thrombin–heparin	5	100,4±2,5 (5)	100,4±2,9 (5)	100,0±1,0 (6)	93,5±1,7* (4)
	15	95,0±1,0 (5)	99,0±4,2 (5)	98,5±1,9 (6)	100,0±4,1 (4)
	30	101,4±3,8 (5)	98,4±2,9 (5)	99,5±1,5 (6)	86,5±7,0 (4)
2 μM Heparin	5	97,7±8,2 (3)	101,0±1,0 (3)	98,0±2,7 (5)	100,0±2,1 (3)
	15	104,6±8,9 (3)	100,0±5,8 (3)	99,2±1,9 (5)	98,7±1,4 (3)
	30	107,0±12,7 (3)	101,3±3,8 (3)	98,6±2,1 (5)	97,3±1,4 (3)
1.8 μM DIP-α-thrombin	5	122,3±8,7* (6)	125,5±8,4* (6)	122,3±6,0* (9)	152,4±9,7* (7)
	15	107,5±2,7* (6)	101,8±4,7 (6)	108,1±3,7* (9)	116,3±7,4* (7)
	30	102,3±3,2 (6)	106,2±2,1 (6)	104,6±1,9* (9)	105,4±3,8 (7)

Legend. Here and in Table 2, number of animals given in parentheses. *P < 0.05 compared with control.

TABLE 2. Changes in Parameters of State of Anticlotting System after Intravenous Injection of Heparin (M ± m)

Parameters of state of anticlotting system	Substance injected		
	0.85% NaCl solution	heparin	
		1,6 μM	4,8 μM
Thrombin time, sec	30,6±0,3 (20)	36,6±4,6* (10)	163,9±14,5* (10)
Nonenzymic fibrinolysis, mm^2	30,6±1,0 (20)	38,7±1,9* (10)	38,1±1,4* (10)
Total fibrinolytic activity, mm^2	39,0±1,3 (20)	46,0±1,7* (10)	49,0±3,3* (10)
Enzymic fibrinolytic activity, mm^2	59,3±4,0 (20)	87,9±4,5* (10)	103,0±6,1* (10)
Plasmin activity, mm^2	8,6±0,6 (20)	8,7±0,3 (10)	8,4±0,6 (10)

In the next series of experiments the carotid sinus zone of rabbits was perfused with the DIP-α-thrombin–heparin complex (2 μM); parameters characterizing the state of the anticlotting system in this case remained at the basal level (Table 1). Investigation of the state of the anticlotting system over a period of 30 min likewise revealed no significant deviations from the initial level. No heparin could be found by the methods used in perfusates of the DIP-α-thrombin–heparin complexes. In a series of control experiments with perfusion of the carotid sinus zone with heparin in molar concentration corresponding to its concentration in the complex with DIP-α-thrombin (2 μM) no change in the parameters of the state of the anticlotting system likewise was observed (Table 1). After intravenous injection of 1.6 or 4.8 μM heparin the change in the parameters of the anticlotting system was expressed as a significant increase in nonenzymic and enzymic fibrinolysis and a sharp increase (by 2.8 times) in thrombin time (Table 2). Comparison of the results obtained by perfusion of the carotid sinus zone of a rabbit with heparin and after intravenous injection of heparin in a closely similar molar concentration points to the absence of any neurohumoral response of the animal to heparin. Changes in the anticoagulant and fibrinolytic background associated with intravenous injection of heparin were due both to complex formation by it with blood proteins and to secretion of a tissue plasminogen activatory by the endothelium in response to the appearance of heparin in the bloodstream [11].

It can be postulated that, by forming a complex with thrombin, heparin blocks the cationic subcenter of the recognition center for high-molecular-weight substrates in the enzyme molecule and disturbs its ability to bind with specific receptors of the vessel wall, thereby preventing manifestation of the response of the anticlotting system.

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ISOLATION, AMINO ACID COMPOSITION, AND BIOLOGICAL ACTION OF A PEPTIDE BIOREGULATOR FROM COW K-CASEIN

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Research workers are currently paying great attention to peptide bioregulators, for these molecules occupy a central position in the system of endocrine regulation of the body. Not only substances traditionally regarded as peptide regulators, but also degradation products of unspecialized precursor proteins, may also exhibit biological activity.

It has been shown [5, 7, 8-10, 12] that the mammalian dietary protein, milk casein, during proteolysis forms a series of physiologically active peptides, capable of regulating the circulation and the secretory and motor functions of the stomach and of exhibiting opium-like activity.

Considering the extraordinary importance of peptide bioregulators as potentially useful therapeutic agents, it was decided to isolate a peptide preparation from k-casein with a distinct biological action and to study its amino-acid composition and physiological activity.

EXPERIMENTAL METHOD

Total casein was obtained from fresh, defatted milk by acid precipitation at the isoelectric point. α-Casein was isolated from total casein by Little's method and purified twice to remove traces of α_s- and β-caseins by precipitation from 50% ethanol with ammonium acetate [16], and dialyzed against 0.5% NaCl. A small fraction (10-20 ml) of the solution obtained after dialysis was freeze-dried and the purity of the α-casein was verified by electrophoresis. The purified α-casein was used to isolate the peptide. For this purpose, the concentration of α-casein was determined spectrophotometrically in the solution obtained after dialysis, on the assumption that $E^{0.1\%}_{280\,nm} = 1.120$, and it was diluted to a 2% concentration with 0.5% NaCl solution, heated to 37°C, after which the pH was adjusted to 5.65 with 0.1 N HCl, and a weighed sample of pepsin, dissolved in a small quantity of distilled water was added (at the rate of one part of enzyme to 100 parts of α-casein by weight). After incubation (2.5 min) a 50%

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