PHYSIOLOGY

The Antithrombotic and Thrombolytic Activity of Tuftsin

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It has been established that one of the immune regulatory peptides, tuftsin, with the amino acid sequence Thr-Lys-Pro-Arg, has a depolymerizing effect on fibrin [2] and prevents its polymerization [5]. It has also been shown that tuftsin displays anticoagulant properties [4], which are related to the sequence Pro-Arg [10]. Normal blood contains around 300 μg/liter of tuftsin [8]. Such a concentration in *in vitro* experiments produces a fibrinolytic effect [4]. Tuftsin may interact *in vitro* with high-molecular heparin, resulting in the formation of a complex [6] possessing anticoagulant and fibrinolytic properties. Interaction with heparin *in vivo* may result in a rise of the anticoagulant and fibrinolytic potential of the blood [4].

The capacity of tuftsin to produce antithrombotic and thrombolytic activity in the organism was examined in the present study.

MATERIALS AND METHODS

Tuftsin synthesized at the University of St. Petersburg was used. The anticoagulant activity of cattle serum was determined by the time of recalcification using the routine method and by the thrombin time [7]. Determination of the fibrinolytic properties of tuftsin was performed using a method described elsewhere [3] on fibrin plates nonstabilized with factor XIIIa. The capacity of tuftsin to lyse nonstabilized fibrin was assessed both in the absence and in the presence of the blocker of enzymatic fibrinolysis ε-aminocaproic acid (in a final concentration of 3%).

In vivo experiments were carried out on 75 male albino rats weighing 180-200 g. The antithrombotic and thrombolytic activity of tuftsin was examined using the following method [11]: 0.5 ml of a 0.01% solution of tuftsin was injected i.v. per 200 g weight of animals (250 μg/kg) 5 min before administration of tissue thromboplastin (in the case of determination of the antithrombotic effect) or 10-15 min after thrombus formation in *v. jugularis* (when the thrombolytic effect was being determined). In the latter case the part of the vein with the thrombus was dissected 4-5 h later, the thrombus was removed, freeze-dried at 37°C for 24 h, and then weighed. Control animals received solvent, namely, 0.85% NaCl instead of tuftsin solution. The results were processed statistically using the Fisher-Student test.

RESULTS

The data obtained previously [4] on the anticoagulant properties of tuftsin *in vitro* were now con-

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TABLE 1. Nonenzymatic Fibrinolytic Activity of Tuftsin *in Vitro* ($M \pm m$)

Experimental conditions	Consentration of drug, mg/ml	SFA, mm²	NF, mm²
Control, 0.85% NaCl solution	–	0	0
Experiment, tuftsin	$10-1$	36±1.1	27±3.3
	10^{-2}	36±1.1	31±4.1
	10^{-3}	42±0.2	32±2.7
	10^{-4}	49±0.9	34±4.1
	10^{-5}	49±1.0	35±3.0
	10^{-6}	49±1.0	35±0.9
	10^{-7}	49±0.9	31±2.2
	10^{-8}	42±2.0	30±1.8
	10^{-9}	42±3.3	31±4.3
	10^{-10}	30±2.1	20±0.2
	10^{-11}	20±0.0	12±0.9
	10^{-12}	0	0

Note. Statistical indexes are reliable ($p<0.001$) relative to the corresponding control samples. SFA: summary fibrinolytic activity; NF: nonenzymatic fibrinolysis.

firmed. Tuftsin in a final concentration of 10^{-1}-10^{-3} mg/ml prolonged the recalcification time of normal serum by 25% on average. In a concentration of 1 mg/ml there was no formation of a clot in the medium after the addition of calcium chloride solution. Examination of the anticoagulant activity of preparations by the test of thrombin time showed a practically insignificant deviation from the control level. This is probably related to the capacity of tuftsin to block the earlier process of thromboplastin formation, but not the process of thrombin formation.

It was established that tuftsin caused lysis of fibrin nonstabilized by factor XIIIa. Zones of lysis on fibrin plates reached 36-40 mm² for the action of 0.03 ml of tuftsin solution containing 3 μg of the drug in the presence of 0.85% NaCl solution (the summary fibrinolytic activity) and 30-34 mm² in the presence of ε-aminocaproic acid (nonenzymatic fibrinolysis). A dose-dependent effect of the preparation was not found in concentrations from 10^{-4} to 10^{-9} mg/ml (Table 1).

In experiments in vivo tuftsin prevented dangerous situations in the organism where thrombosis was provoked by i.v. administration of tissue thromboplastin. The antithrombotic effect of tuftsin in a dose of 50 μg/200 g weight was statistically reliable in all test animals ($p<0.05$) and was 80% on average as compared to the control. Among 7 control rats 5 developed shock, whereas the administration of tuftsin prior to injection of thromboplastin prevented the development of shock. The autopsy of shocked control animals revealed massive thrombosis of the pulmonary arteries. There were no thrombi in vessels of internal organs in the test animals. Since tuftsin has a proven antithrombotic effect, it was assumed that it may exhibit a thrombolytic effect and lyse fresh experimentally produced thrombi.

As is evident from Table 2, tuftsin in a rather low dose (50 μg/200 g weight) may produce a thrombolytic effect, judging from the decrease of thrombus weight (by 26%) in test animals treated with tuftsin as compared to the control.

Thus, tuftsin displays both anticoagulant and nonenzymatic fibrinolytic properties *in vitro* and produces *in vivo* both an antithrombotic and a thrombolytic effect on freshly produced thrombi. Tuftsin is likely to be specified as a preparation of fibrinolytic and antithrombotic action that may be of certain practical interest, since the drug exhibits other useful properties (such as its stimulation of antiinfectious defense [9], adaptogenic effect [1], and anticoagulant effect [2]).

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TABLE 2. Antithrombotic and Thrombolytic Activity of Tuftsin in a Dose of 50 μg/200 g Weight of Rats in i.v. Administration

Experimental conditions	Antithrombotic effect, weight of thrombi, g ($n=12$)	Thrombolytic activity, weight of thrombi, g ($n=7$)
Control, administration of NaCl solution (0.5 ml/200 g)	2.9±0.01	15.5±0.02
Experiment, administration of tuftsin 50 μg/200g	2.15±0.24	12.5±0.6

Note. Statistical indexes are reliable ($p<0.05$) relative to control values.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

The Effect of Catecholamine and Serotonin Antibodies on Pain Sensitivity and on the Development of Morphine Tolerance in Experimental Narcomania

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Antibodies (AB) against the neurotransmitters serotonin (5-HT), dopamine (DA), and norepinephrine (NE) [2,6,8,9,12] are found in different forms of CNS pathology (parkinsonism, alcoholism, narcomania, PKU) and in hypertension, and possess a broad spectrum of biological activity, namely, they change behavior reactions of animals [1,16], inhibit alcohol motivation [4,7], and may provoke (AB against DA) or attenuate (AB against 5-HT) the development of Parkinson's syndrome when locally administered to brain structures [11,13]. Chronic morphinization of animals induces the production of autoAB against neurotransmitters (5-HT and cathecholamines), as was demonstrated previously [3]. AutoAB against neurotransmitters were also found in the blood serum of patients with different types of narcotic dependence [2]. Published data attest to the participation of the catecholamine- and 5-HT-ergic systems in the correction of pain and in the mechanisms of the analgesic effects of morphine [10,19,22,23], as well as in the development of morphine tolerance [5].

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