PHYSIOLOGY

Anticoagulant Activity and Platelet Aggregation in Blood Plasma from Rats after Intranasal Administration of Adenosine Triphosphate and Its Complex with Heparin

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Fourfold intranasal administration of ATP was followed by an increase in anticoagulant activity and decrease in platelet aggregation in blood plasma of healthy rats and, particularly, of animals with suppressed function of the anticoagulant system. These changes decrease the risk of the prethrombotic state. Complex of ATP and high-molecular-weight heparin decreased platelet aggregation only in the blood from healthy animals, but increased anticoagulant potential of plasma hemostasis in prethrombotic animals.

Key Words: *adenosine triphosphate-heparin complex; anticoagulant activity; platelet aggregation*

ATP is a natural high-molecular-weight ligand formed during glycolytic degradation of carbohydrates [9]. Anticoagulant effect of ATP were demonstrated i*n vitro* and *in vivo* by computer modeling and in experimental coagulation studies [5,6,8]. Another blood anticoagulant, heparin, is present in the vascular endothelium and blood, is highly reactive to the formation of complexes with various substances [10], including ATP [5]. Our previous studies showed that a complex of ATP and heparin (ATP-H) demonstrates strong anticoagulant, fibrin-depolymerizing, and fibrinolytic effects and reduces ADP-induced platelet aggregation *in vitro* and after intramuscular injection to healthy animals [5,7]. Since parenteral treatment with these preparations causes side effects, they are often administered intranasally. After intranasal administration, this drug enters the blood flow, reaches the Jacobson's organ, and contributes to transduction of nerve impulses to brain receptors [2]. The anticoagulant system is of the neurohumoral nature [4]. Aging of the organism is accompanied by a decrease in anticoagulant function [1]. It remains unclear whether intranasal administration of ATP and ATP-H can correct plasma and platelet hemostasis in animals of different age.

This work was designed to study anticoagulant activity of the plasma and platelet hemostasis in animals of different age after intranasal administration of ATP and ATP-H.

MATERIALS AND METHODS

Experiments were performed with 1% ATP sodium salt. ATP-H was obtained as described elsewhere [5].

Male laboratory rats were *in vivo* divided into 3 groups. Group 1 consisted of healthy adult animals aging 4-4.5 months and weighing 190-210 g.

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Groups 2 and 3 included rats aging 8-8.5 (350-380 g) and 10 months (400-430 g), respectively.

The test preparations were administered intranasally at 24-h intervals (4-fold treatment). Each group of rats was divided into the following subgroups: subgroup A, administration of NaCl (control); subgroup B, administration of ATP; and subgroup C, administration of ATP-H. The blood was taken from the jugular vein with a syringe with 3.8% sodium citrate (blood/anticoagulant ratio 9:1). Blood sampling was performed 1-1.25 h after the last treatment with the test preparations. The blood was centrifuged to study platelet aggregation (1000*g*, 5.5 min) and blood coagulation (3000*g*, 12 min).

Blood anticoagulant activity was estimated from activated partial thromboplastin time (APTT) [3]. Platelet aggregation was evaluated in platelet-rich plasma after addition of the aggregation-inducing agent (2-5 μM ADP) by measuring optical density of plasma samples on an aggregometer (M. V. Lomonosov Moscow State University). Platelet aggregation was expressed as an index of the light scattering increment (ISL) and in percent of the control value.

The results were analyzed by Student's *t* test.

RESULTS

Table 1 shows parameters of platelet aggregation and anticoagulant activity of blood plasma in rats of various experimental groups after 4-fold intranasal administration of ATP, ATP-H, and NaCl.

Intranasal administration of ATP (0.96 mg/kg, 0.02 ml) to healthy adult animals was followed by a slight increase in anticoagulant activity (by 10%) and decrease in platelet aggregation (by 13%) compared to the control. Similar changes were previously revealed after intramuscular injection of ATP to healthy rats [8]. Intranasal administration of ATP-H (1 mg/kg) induced a significant decrease in platelet aggregation (by 48%) and increase in anticoagulant activity of blood plasma (by 1.14 times) in animals of this group (Table 1).

Intranasal administration of ATP to 8-8.5-month-old rats with partial suppression of the anticoagulant system (ACS) was followed by a significant decrease in platelet aggregation (by 49% below the control, Table 1) [1,4]. Administration of ATP-H under these conditions had no effect on platelet aggregation (in contrast to ATP). Anticoagulant activity of blood plasma increased by 1.16 times after treatment with ATP and ATP-H.

Intranasal administration of ATP and ATP-H had a similar effect on platelet aggregation in group 3 animals with severe dysfunction of ACS. The prethrombotic state in these rats manifested in a decrease in anticoagulant and fibrinolytic activity of the blood and increase in fibrinogen concentration and platelet aggregation [1]. ATP administration to these rats was followed by a significant decrease in platelet aggregation (by 35% compared to the control). However, platelet aggregation remained unchanged after administration of ATP-H. Anticoagulant activity increased by 1.3 and 1.4 times after administration of ATP and ATP-H, respectively.

Analysis of the experimental data on changes in some parameters of plasma and platelet hemo-

TABLE 1. Platelet Aggregation and Anticoagulant Activity of Blood Plasma from Healthy Rats and Animals with Hypofunction of ACS after Intranasal Administration of ATP and ATP-H

Group, age	Platelet aggregation		APTT	
	ISL	%	sec	%
1, 4-4.5 months				
A: administration of NaCl	21.6±2.5	100.0	27.1±1.23	100.0
B: administration of ATP	18.8±3.4	87.1	29.1±1.18	110.0
C: administration of ATP-H	11.3±3.1	52.2	30.6±1.35	114.0
2, 8-8.5 months				
A: administration of NaCl	21.0±2.2	100.0	24.8±0.08	100.0
B: administration of ATP	10.9±5.0**	51.2	28.8±0.61*	116.1
C: administration of ATP-H	21.3±6.7	100.0	28.8±1.0*	116.0
3; 10 months				
A: administration of NaCl	23.9±3.5	100.0	27.1±0.8	100.0
B: administration of ATP	15.5±3.5*	64.8	35.8±0.6*	132.5
C: administration of ATP-H	22.4±6.1	97.1	38.4±1.41*	141.0

Note. *$p<0.05$ and **$p<0.01$ compared to the control (subgroup A).

stasis in rats after intranasal administration of ATP revealed a significant decrease in platelet aggregation in the blood of rats with normal and, particularly, with suppressed function of ACS. These changes became more significant in elder animals. Administration of ATP-H significantly decreased platelet aggregation only in healthy animals. In rats with suppressed function of ACS (8-8.5- and 10 month-old animals), ATP-H did not decrease platelet aggregation. Administration of ATP-H exhibiting high anticoagulant activity *in vitro* [5] is followed by its release into the blood, which contributes to a significant increase in anticoagulant activity. Our results indicate that intranasal administration of ATP to animals with suppressed function of ACS and prethrombotic state decreases the degree of prethrombosis by reducing the level of platelet aggregation, one of the major factors for thrombosis. Intranasal administration of ATP-H increases anticoagulant potential of plasma hemostasis in prethrombotic animals, which decreases the risk of thrombosis.

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