PHYSIOLOGY

Correction Effect of ATP on Platelet Aggregation and Blood Coagulation *In Vitro* and in the Presence of Circulating Thrombin in Rats

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ATP added to plasma samples in concentrations of 5×10^{-3}-5×10^{-5} M *in vitro* decreased ADP-induced platelet aggregation. Platelet aggregation stimulated with thrombin under similar experimental *in vitro* conditions significantly decreased in the presence of 5×10^{-6} M ATP and tended to decrease under the influence of ATP in concentrations of 5×10^{-3} and 5×10^{-7}-5×10^{-9} M ATP. When endogenous thrombin in the circulation was stimulated by intravenous infusion of tissue thromboplastin, pretreatment with ATP (4 intramuscular injections, 0.75 mg/kg) produced a correction effect and normalized disturbed anticoagulant activity and platelet aggregation.

Key Words: *adenosine triphosphate; thrombin; anticoagulant activity; platelet aggregation*

Our previous experiments confirmed anticoagulant activity of some low-molecular-weight plasma ligands, including ATP. These results were obtained in a complex study by computer modeling and *in vitro* tests for coagulation of animal blood. Anticoagulant activity of ATP is related to the interaction of this ligand with calcium ions in blood plasma [3]. Activated partial thromboplastin time (APTT) test *in vitro* showed that the rate of rat plasma coagulation in the presence of ATP and Ca^{2+} decreased by more than 2 times compared to the control. Addition of ATP in concentrations of 5×10^{-3}-5×10^{-5} M to rat platelet-rich plasma significantly decreased ADP-induced aggregation [5]. These data on the decrease in ADP-induced platelet aggregation in the presence of ATP is consistent with the results obtained *in vitro* on mouse blood plasma [6]. As distinct from ADP, ATP can be considered as an antagonist of purine receptors $R2Y_{12}$ and $R2Y_1$. The generation of phosphate nucleotide derivatives by cell ectonucleotidases is blocked in the presence of ATP.

Repeated intramuscular injections of ATP produce a specific effect on the hemostasis system in animals [2,5]: apart from APTT prolongation, we observed stimulation of enzymatic and nonenzymatic fibrinolysis, activation of tissue plasminogen activator, and inhibition of platelet hemostasis (*i.e.*, decrease in platelet aggregation) in blood plasma from healthy rats.

Experiments with ATP administration to animals raise some questions, in particular whether ATP interacts with other (except ADP) aggregation agonists, *e.g.* thrombin, and whether it modulates blood coagulation during thrombin generation.

Here we compared platelet function during *in vitro* stimulation of aggregation with various agonists (primarily ADP and thrombin) in the presence of ATP. In addition, anticoagulant activity of blood plasma during stimulation of endogenous thrombin generation in the blood by injection of tissue throm-

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boplastin should was evaluated *in vivo* in animals receiving ATP.

MATERIALS AND METHODS

ATP sodium salt was used in *in vitro* experiments and for intramuscular injection to animals. Plasminogen-free thrombin (basal activity 22 NIH/mg protein, Kaunas) served as an aggregant in *in vitro* studies. Tissue thromboplastin was routinely prepared from rat brain free from blood vessels (emulsion containing 1 g brain tissue minced in 14 ml 0.85% NaCl). Thromboplastin (coagulant activity 40 sec, 0.4 ml) was injected intravenously after precipitation to stimulate endogenous generation of thrombin. Experiments were performed on 50 male rats weighing 350-400 g.

Plasma coagulation time in blood samples from treated rats was estimated by the APTT test [1]. Platelet aggregation in platelet-rich plasma was measured on an aggregometer (constructed in Moscow State University) by light scatter increment using ADP (5 μM) and thrombin (0.3-0.5 NIH/mg protein) as aggregants and expressed in percents. This parameter in control samples was taken as 100%.

The blood was taken from the jugular vein using a syringe with sodium citrate (anticoagulant/blood ratio 1:9). Platelet-rich and platelet-poor plasma was obtained by centrifugation under various conditions. The results were analyzed by Fischer—Student test.

RESULTS

In *in vitro* experiments ATP in concentrations of 5×10^{-3}-5×10^{-9} M was added to platelet-rich rat plasma samples 3-5 min before addition of aggregants ADP, thrombin, and collagen. ADP-induced platelet aggregation significantly decreased in plasma samples containing ATP in concentrations of 5×10^{-3}, 5×10^{-4}, and 5×10^{-5} M (Table 1). After addition of thrombin in a concentration of 0.3-0.5 NIH/mg protein to samples containing ATP no increase in platelet aggregation above the control level was noted. In samples containing ATP in a concentration of 5×10^{-6} M, platelet aggregation was 20% lower than in the control; in samples containing 5×10^{-5}, 5×10^{-8}, and 5×10^{-9} M ATP, the degree of aggregation tended to decrease (by 10-12%). ATP in a concentration of 5×10^{-7} M significantly increased collagen-induced platelet aggregation.

During stimulation of aggregation with the enzyme thrombin and ADP, ATP in relatively high concentrations *in vitro* did not increase aggregation compared to the control (Table 1). ATP in a concentration of 5×10^{-6} M significantly inhibited thrombin-induced platelet aggregation.

ATP was injected intramuscularly in a dose of 0.75 mg/400 g 4 times at 24-h intervals. Controls received injections of 0.85% NaCl in an equivalent volume. Tissue thromboplastin (coagulant activity 40 sec) was injected intravenously 1.5 h after the last administration of ATP to stimulate thrombin formation in the blood. Study on this model of endogenous thrombin generation showed that the coagulant enzyme is produced over the first 20-40 sec after thromboplastin injection. Thrombin in healthy animals is completely inactivated 10-15 min after its generation [4].

Administration of ATP to rats significantly prolonged APTT and decreased ADP-induced platelet aggregation, which is consistent with the results of our previous experiments [5]. APTT in the blood

TABLE 1. Platelet Aggregation in Rat Blood Plasma after Addition of ATP in Various Concentrations and Subsequent Stimulation of Aggregation with ADP, Thrombin, and Collagen (%, *M*±*m*)

ATP concentration, M	Aggregant		
	ADP	thrombin	collagen
Control	104.5±11.2	101.5±8.9	103.4±4.8
5×10^{-3}	43.5±7.7**	90.5±4.5	90.0±5.8
5×10^{-4}	45.2±6.8**	103.5±4.4	121.0±7.1*
5×10^{-5}	7.3±9.0*	88.0±4.8	106.2±6.7
5×10^{-6}	95.5±11.0	80.1±4.0*	117.0±7.5
5×10^{-7}	117.0±6.6	94.3±5.0	108.0±5.3
5×10^{-8}	100.0±4.3	86.6±5.1	—
5×10^{-9}	117.0±6.9	87.0±5.9	—

Note. *$p<0.05$ and **$p<0.01$ compared to the control.

TABLE 2. Platelet Aggregation and Anticoagulant Activity (APTT Test) in Blood Plasma from Rats Receiving Intravenous Injection of Tissue Thromboplastin after 4-fold Administration of ATP

Group	Parameters of hemostasis before thromboplastin injection		Parameters of hemostasis after thromboplastin injection			
	APTT, sec	aggregation index, %	APTT, sec		aggregation index, %	
			after 1 min	after 7-9 min	after 1 min	after 7-9 min
Control	31.0±2.0	23.7±4.0	25.5±1.5*	38.6±4.0	8.5±3.8** (32% of normal)	13.2±3.7* (50% of normal)
Treatment	40.7±1.9*	19.1±1.7	29.2±1.1	59.6±3.9**	6.8±2.3** (26.2% of normal)	18.8±2.5 (72.2% of normal)

Note. *$p<0.05$ and **$p<0.01$ compared to the control (before thromboplastin injection).

from control and treated animals decreased over the 1st minutes after thromboplastin injection, which coincides with the maximum generation of circulating thrombin. Seven-nine minutes after treatment, blood coagulation time (APTT test) in treated rats considerably increased in comparison with the corresponding value in the control group. This period corresponds to inactivation of thrombin in the circulation. Table 2 shows that ADP-induced platelet aggregation in animals of both groups decreased similarly during the maximum generation of endogenous thrombin (1st minute after thromboplastin injection). ADP-induced aggregation in treated rats returned to normal during the follow-up period.

The results of *in vitro* and *in vivo* studies indicate that circulating ATP not only decreases coagulant activity of enzyme thrombin and normalizes blood coagulation, but also provides rapid correction of ADP-induced aggregation to normal values.

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