Anticoagulation and Antiplatelet Effects of Semax under Conditions of Acute and Chronic Immobilization Stress

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The effects of semax on anticoagulant, fibrinolytic, and platelet components of the anticoagulation system were studied on albino rats under conditions of acute and chronic immobilization stress. Semax exhibited a protective antistress effect after repeated intranasal administration under conditions of hypercoagulation developing in response to immobilization stress of different degree. The effect manifested in stimulation of the anticoagulation system.

Key Words: *semax; glyprolines; fibrinolysis; blood anticoagulant activity; platelet aggregation*

Simple proline-containing peptides exhibit anticoagulant, fibrinolytic, and antiplatelet effects in the blood stream, stimulating the function of the blood anticoagulation system [3,6]. On the other hand, many regulatory peptides are characterized by wide spectra of biological activities and among other things are involved in the maintenance of normal homeostasis during stress [1,2]. The antistress effects of Pro-Gly-Pro, Pro-Gly, and Gly-Pro glyprolines and $ACTH_{4-10}$ fragment of semax in various types of stress were demonstrated [12].

It is known that the development of adaptive reactions can be associated with stimulation or inhibition of some components of hemostasis (depending on the duration and intensity of stress) [7,13]. Short (<30 min) immobilization stress leads to stimulation of the anticoagulation system and appearance of nonenzymatic fibrinolysis agents [10], *e.g.* glyprolines [1,6] in the blood. Longer stress (*e.g.*, immobilization stress for 60-90 min) activates the platelet component of the hemostasis and inhibits fibrinolytic and anticoagulant activities of the blood, which stimulates blood clotting [14].

It is just natural to suggest in this case that administration of proline-containing peptides under conditions of hypercoagulation during immobilization stress will stimulate the function of the blood anticoagulation system. Understanding of the mechanisms of regulation and work of the hemostasis system under the effects of regulatory peptides opens new vistas for maintaining high adaptation potentials under conditions of stress.

We studied the effects of semax on the anticoagulant, fibrinolytic, and platelet components of the blood anticoagulant system in experimental animals under conditions of acute and chronic immobilization stress.

MATERIALS AND METHODS

Russian commercial peptide drug Met-Glu-His-Phe-Pro-Gly-Pro (semax) was used in the study.

Experiments were carried out on 77 8-9-month-old male albino rats (200-250 g) kept on common laboratory diet. The animals received intranasal semax (0.05 ml; 1 mg/kg; experimental group) or 0.05 ml 0.85% NaCl solution (control group) every 24 h for 4 days. The blood for analysis of the hemostasis parameters was collected from the jugular vein with a syringe with 3.8% sodium citrate (9:1 blood:citrate ratio).

Anticoagulant parameters of the plasma were evaluated: activated partial thromboplastin time (APTT) [5], fibrinolytic activities – summary (SFA) and non-

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enzymatic (NEFA) [10] (using unstabilized fibrin), activity of plasminogen tissue activator (PTA) on warmed and not warmed stabilized fibrin plates [4], ADP-induced aggregation of platelet-rich plasma [5].

Two series of animal experiments were carried out. In series I, the protective antistress effect of semax was evaluated under conditions of acute immobilization stress. The rats were intranasally administered the peptide (or 0.85% NaCl) and after the last dose of the drug (or NaCl) the animals were exposed to immobilization stress (fixation in the supine position for 60 min). In experimental series II, the protective antistress effect of semax was evaluated under conditions of chronic immobilization stress. Similarly to series I, the rats received intranasal semax (or 85% NaCl) and 1 h after each dose were daily subjected to 60-min immobilization stress. Blood for analysis was collected 1 h after the start of immobilization following peptide administration (experiment) or 0.85% NaCl administration (control).

The data were statistically processed using Student's test and nonparametric tests.

RESULTS

Previous experiments on intact rats showed that anticoagulant, fibrinolytic, and antiaggregant activities of the blood increased 1 h after 4 daily doses of intranasal semax (1 mg/kg) [6]. These results suggested that semax could be useful for correction of hypercoagulation disorders in the hemostasis system, specifically, in immobilization stress of a certain duration (60-90 min).

Experiments on rats exposed to 60-min immobilization stress (single and 4 daily sessions) showed that this stress causes hypercoagulation and hypofibrinolysis because of intensification of platelet aggregation under the effect of ADP and led to a decrease in plasma anticoagulant and fibrinolytic activities of nonenzymatic and enzymatic nature. Chronic immo-

TABLE 1. Parameters of Rat Plasma during 60-min Immobilization Stress after Four Intranasal Semax Doses (1 mg/kg; *M*±*m*)

Experiment conditions	Parameter, %					
	APTT	SFA	NEFA	EFA	PTA	Platelet aggregation
Normal values (intact rats)	100.0±10.2	100.0±13.4	100.0±13.7	100.0±6.2	100.0±14.5	100.0±21.2
Immobilization after 0.85% NaCl (control)	70.0±5.1*	81.0±3.5	77.7±1.6*	89.8±5.3*	98.7±2.9	108.0±10.6
Immobilization after semax (experiment)	105.0±10.8^{++}	138.0±14.4^{++}	118.0±12.4^{+}	142.8±19.3^{*+}	219.4±32.2^{*++}	72.3±7.7^{+}

Note. Here and in Table 2: data expressed in percents of normal values (taken for 100%). *p<0.05, **p<0.01 compared to normal; ^{+}p<0.05, ^{++}p<0.01 compared to the control.

TABLE 2. Parameters of Rat Plasma after Development of Chronic (4-Day) Immobilization Stress during Intranasal Semax Treatment (4 Doses of 1 mg/kg; *M*±*m*)

Experiment conditions	Parameter, %					
	APTT	SFA	NEFA	EFA	PTA	Platelet aggregation
Normal values (intact rats)	100.0±2.2	100.0±6.1	100.0±7.3	100.0±5.2	100.0±14.6	100.0±16.1
Immobilization after 0.85% NaCl (control)	79.2±5.5*	81.8±6.2	90.1±7.4	71.8±3.4*	96.7±7.6	154.7±17.4*
Immobilization after semax (experiment)	105.7±5.8^{+}	133.2±10.6^{*++}	129.2±9.6^{+}	121.2±7.4^{+}	209.4±20.9^{*++}	124.0±12.8

bilization stress was associated with a more significant increase in platelet aggregation and reduction of enzymatic fibrinolysis, while single 60-min immobilization exposure led mainly to a decrease in anticoagulant activity of the blood and nonenzymatic fibrinolysis (Tables 1, 2).

In subsequent experiments, the effects of intranasal semax on changes in the hemostasis system of rats subjected to immobilization stress of different intensity were studied.

Intranasal administration of semax for 4 days prevented the development of hypercoagulation after single and chronic (60 min per day for 4 days immobilization stress. This was not paralleled by reduction of blood anticoagulant activity. ADP-induced platelet aggregation increased negligibly in chronic stress (by 24% in comparison with intact rats) or decreased to 72.3% of the control level after single exposure. As for changes in the fibrinolysis system, plasma SFA and NEFA values increased by 33-38 and 18-29%, respectively, after immobilization stress of different intensity after semax administration in comparison with normal hemostasis values (Tables 1 and 2).

In addition, semax treatment led to a 21% increase in enzymatic fibrinolytic activity (EFA) under conditions of chronic stress and a 42.8% increase after acute stress in comparison with the values in intact rats; EFA decreased significantly after saline instead of semax. EFA increased at the expense of a significant (more than 2-fold) increase in PTA activity (Tables 1, 2).

Hence, our results indicate that single or daily (for 4 days) immobilization stress promotes inhibition of the anticoagulation system function and stimulates blood clotting. Chronic immobilization stress is associated with a more pronounced decrease of enzymatic fibrinolysis and increase of platelet aggregation, while single 60-min immobilization leads to a more pronounced reduction of the blood anticoagulant activity and nonenzymatic fibrinolysis. Intranasal semax treatment under conditions of immobilization stress of different severity resulted in an increase of blood fibrinolytic activity (nonenzymatic and enzymatic) and PTA activity. This indicated that semax induced the endothelium-dependent reaction of plasminogen activator release into the blood stream. Repeated intranasal semax administration was not associated with reduction of plasma anticoagulant activity after immobilization stress, due to inhibition of thrombin activity by the regulatory peptides [8,9]. In addition, ADP-induced platelet aggregation in chronic stress under these conditions increased negligibly or even decreased after a single immobilization exposure, due to peptide inhibition of thrombin activity and their interference in the platelet aggregation processes [11].

Hence, repeated administration of semax promotes an increase in plasma anticoagulant, fibrinolytic, and antiaggregation activities and protects animals from the hypercoagulation effects of 60-min immobilization, both acute and chronic. These effects of semax can be explained by its capacity to increase antiaggregation, anticoagulant, and fibrinolytic activities of enzymatic and nonenzymatic nature. These effects of semax extend the sphere of its application as a drug protecting from hypercoagulation during stressogenic exposure.

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