Changes in Hemostatic Parameters after Intranasal Administration of Peptide Pro-Gly-Pro

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Anticoagulant activity, total and nonenzymatic fibrinolytic activity, and tissue plasminogen activator activity increased, while platelet aggregation and activity of factor XIIIa in rat blood plasma decreased over 3 h after single intranasal administration of proline-containing peptide Pro-Gly-Pro. Hemostatic parameters in rats 24 h after single administration of this peptide did not differ from the control level observed in animals receiving 0.85% NaCl.

Key Words: *peptide Pro-Gly-Pro; hemostatic system; fibrinolysis; platelet aggregation*

Glyprolines, regulatory proline-containing peptides, modulate several processes in the organism, play a role in inhibition of blood coagulation, normalize impaired cerebral circulation, and stimulate nerve regeneration [1,2,10]. Peptides Pro-Gly-Pro and Pro-Gly produce an antithrombotic effect [7], which is related to antiplatelet, anticoagulant, and fibrinolytic activities. Anticoagulant, fibrinolytic, and antiplatelet activities of blood plasma increase over the first minutes and remain high for 1-2 h after intravenous injection of peptide Pro-Gly-Pro. Antiplatelet, anticoagulant, and fibrinolytic activities of the plasma in rats increased 1 h after intraperitoneal and peroral administration of Pro-Gly-Pro [5]. Previous experiments with a radioactive label showed that [^{3}H]Pro-Gly-Pro was present in the circulation for 5 h after single intraperitoneal injection [3].

Here we studied the dynamics of the anticoagulant effect of Pro-Gly-Pro after intranasal administration.

MATERIALS AND METHODS

Experiments were performed with a short proline-containing peptide Pro-Gly-Pro synthesized at the Institute of Molecular Genetics (Moscow).

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Total (TFA) and nonenzymatic (NEFA) fibrinolytic activity, platelet aggregation, and anticoagulant activity were studied *in vitro* after administration of the peptide in concentrations of 10^{-4}-10^{-11} M.

In vivo experiments were performed on 48 male laboratory rats aging 5.5-6.0 months and weighing 200-220 g. The animals were divided into 2 groups. Peptide Pro-Gly-Pro (0.05 ml, 1 μg/kg) was administered intranasally to group 1 rats (treatment). Group 2 animals received an equivalent volume of 0.85% NaCl (control). The jugular vein blood (1.5 ml) was taken from each rat 1, 2, 3, 5, and 24 h after peptide treatment to evaluate biochemical parameters of hemostasis. The solution of sodium citrate (3.8%) served as an anticoagulant. The following parameters of coagulant and anticoagulant plasma systems were evaluated: TFA, NEFA [9], tissue plasminogen activator activity [8], and anticoagulant activity (test for activated partial thromboplastin time, APTT) [4]. Platelet aggregation in the plasma was studied on an aggregometer. The solution of ADP in a concentration of 2 μM served as ggregation induction [4]. Activity of factor XIIIa was measured as described elsewhere [4].

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

RESULTS

The peptide preparation *in vitro* exhibited TFA, which was related to NEFA (fibrin-depolymerizing

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activity). The lysis zone was 30-33 mm^2. Addition of this peptide in concentrations of 10^{-4}-10^{-11} M to rat plasma samples produced anticoagulant and antiplatelet effects (22% increase in APTT and 30-40% decrease in platelet aggregation).

Experiments on animals revealed an increase in plasma anticoagulant activity (by 1.3 times, APTT test), TFA and NEFA (by 1.5 times), and tissue plasminogen activator activity (by 2 times) 1 h after intranasal administration of the peptide. Activity of factor XIIIa in treated animals decreased by 18 U/ml compared to the control (statistically insignificant).

APTT of blood plasma approached the control level 2 h after single intranasal administration of Pro-Gly-Pro. During this period, TFA, NEFA, and tissue plasminogen activator activity exceeded the control level by 1.4, 1.34, and 1.4 times, respectively. Platelet aggregation in blood plasma from treated animals decreased by 39.5%. Activity of factor XIIIa in blood plasma from treated animals during this period did not differ from the control level.

APTT in rat blood slightly increased by the 3rd hour after intranasal administration of Pro-Gly-Pro (statistically insignificant). TFA and NEFA in treated animals remained elevated in this period (1.3 times higher compared to control rats). Activity of tissue plasminogen activator in rats of the treatment group surpassed the control level by 25%. Platelet aggregation in the blood of treated rats did not differ from the control (Table 1).

Five hours after intranasal administration of the peptide, TFA and NEFA of blood plasma were elevated by 1.23 times. Activity of tissue plasminogen activator in treated rats was 1.3-fold surpassed control. During this period, APTT did not differ from the control.

APTT, TFA, platelet aggregation, and activity of factor XIIIa in rats 24 h after intranasal administration of the peptide did not differ from those in control animals. However, NEFA and tissue plasminogen activator activity in treated rats were higher compared to the control (Table 1).

Studying the dynamics of hemostatic parameters after intranasal administration of Pro-Gly-Pro showed that the anticoagulant effect of this peptide developed over the first minutes after treatment [2] and persisted for 3 or even 5 h. Hemostatic parameters returned to normal 24 h after administration of the peptide. Peptide-induced changes in the enzymatic and nonenzymatic fibrinolytic systems were most persistent. Antiplatelet and anti-fibrin-stabilizing activity decreased to normal 3 h after peptide administration. The mechanisms for anticoagulant and fibrinolytic activity of Pro-Gly-Pro and Pro-Gly-Pro-containing peptide Semax were compared. It should be emphasized that Semax produces an indirect effect. However, intranasal administration of Pro-Gly-Pro produced a direct effect.

Our study showed that intranasal administration of peptide Pro-Gly-Pro produced a long-lasting anti-

TABLE 1. Hemostatic Parameters in Blood Plasma of Rats after Intranasal Administration of Peptide Pro-Gly-Pro in a Single Dose of 1 mg/kg ($M\pm m$)

Group, parameter	Time after Pro-Gly-Pro administration, h				
	1	2	3	5	24
Control					
APTT, sec	46.1±4.3	45.0±4.3	45.3±4.9	45.0±4.0	46.3±5.4
TFA, mm^2	34.4±2.7	32.3±2.2	34.8±2.3	34.0±2.1	33.5±2.0
NEFA, mm^2	22.6±2.3	23.0±0.9	23.0±1.3	23.0±0.8	23.0±1.6
PAA, %	100.0±1.9	100.0±2.9	100.9±2.4	100.6±8.0	102.0±1.7
Factor XIIIa, U/ml	83.1±6.9	100.0±7.3	—	—	102.0±1.5
Platelet aggregation, %	101.0±4.0	100.5±5.4	100.5±7.5	—	—
Treatment					
APTT, sec	63.1±4.4*	49.0±8.5	53.3±6.9	49.7±4.0	48.3±2.4
TFA, mm^2	51.4±3.2**	50.3±2.2**	48.8±3.3**	39.0±1.1*	34.0±2.5
NEFA, mm^2	34.8±1.3**	31.0±1.4*	31.2±1.7*	29.3±0.8	28.0±1.7
PAA, %	200.0±11.5**	140.0±8.9**	125.9±5.4	130.6±8.7*	104.0±3.7
Factor XIIIa, U/ml	52.1±4.0**	94.0±4.0	—	—	—
Platelet aggregation, %	101.0±14.0	60.5±5.4**	132.5±17.5	—	100±4.5

Note. PAA, tissue plasminogen activator activity; —, not measured. *$p<0.05$ and **$p<0.01$ compared to the control.

coagulant effect, which persisted for up to 5 h. However, antiplatelet activity of blood plasma returned to normal 3 h after peptide administration. Hence, Pro-Gly-Pro improved rheological properties of the blood. Moreover, this peptide produced no side effects. These data extend the approaches to the use of Pro-Gly-Pro in clinical practice.

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