Comparative Study of Modulatory Effects of Semax and Primary Proline-Containing Peptides on Hemostatic Reactions

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Intranasal administration of Semax, peptide Pro-Gly-Pro, and a mixture of peptides Pro-Gly+Gly-Pro to rats for 5 days enhanced anticoagulant and fibrinolytic potential of the plasma (total fibrinolytic activity and plasmin and plasminogen activator activities) and decreased antiplasmin concentration. Semax and Pro-Gly-Pro decreased the weight of thrombi during experimental thrombosis.

Key Words: *hemostasis; proline-containing peptides; Semax*

Primary proline-containing peptides Pro-Gly, Gly-Pro, and Pro-Gly-Pro, fragments of collagen and elastin, inhibit blood coagulation and formation of thrombi [2-4,7]. The tripeptide Pro-Gly-Pro is the most stable *in vivo* fragment of nootropic peptide Semax (Met-Gly-His-Phe-Pro-Gly-Pro), which holds much promise for combination therapy of ischemic stroke [6]. The question arises as to whether Semax can modulate hemostatic reactions (similarly to Pro-Gly-Pro). Moreover, it remains unclear whether the ameliorating effects of Semax in stroke are related to the *in vivo* release of tripeptide Pro-Gly-Pro.

Here we compared *in vivo* effects of Semax, tripeptide Pro-Gly-Pro, and a mixture of dipeptides Pro-Gly and Gly-Pro on parameters of hemostasis in rats.

MATERIALS AND METHODS

Experiments were performed on outbred male rats weighing 200-250 g. Pro-Gly-Pro (1 mg/kg), Semax (1 mg/kg), and a mixture consisting of 0.5 mg/kg Pro-Gly and 0.5 mg/kg Gly-Pro (both from Sigma) were administered intranasally for 5 days. Control rats intranasally received an equivalent volume of physiological saline. Hour and a half after the last treatment (day 5) the blood from the jugular vein was collected into a plastic tube with 3.8% sodium citrate (9:1) and centrifuged at 1800 rpm for 10 min.

Semax and tripeptide Pro-Gly-Pro were synthesized at the Laboratory of Regulatory Peptides (Institute of Molecular Genetics).

Hemostasis parameters were measured by routine biochemical methods. Plasma fibrinogen concentration was measured by the method described previously [1] with modifications. Fibrinolytic activity (FA) on standard fibrin plates and plasminogen activator (PA) activity of plasma euglobulin fraction were estimated as described elsewhere [8]. Antiplasmin activity was measured using a chromogenic substrate s-2251 [9]. Anticoagulant activity (AA) was evaluated by activated partial thromboplastin time [5]. Thrombosis was induced as described previously [10]. The results were analyzed by Student's *t* test.

RESULTS

The time of clot formation in the plasma of control animals was 41.1±2.78 sec. Intranasal administration of tripeptide Pro-Gly-Pro, dipeptide mixture Pro-Gly and Gly-Pro, and Semax for 5 days increased AA by 1.5, 1.4, and 1.7 times, respectively, compared to the control ($p<0.001$). Hence, Semax possessed most potent anticoagulant properties.

In animals receiving test peptides the parameters of the fibrinolytic system also increased to a different extent (Table 1). The Pro-Gly+Gly-Pro mixture pro-

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TABLE 1. Effects of Proline-Containing Peptides on Parameters of the Plasma Fibrinolytic System in Experimental Animals (*M*±*m*)

Parameter	Control (*n*=15)	Semax (*n*=14)	Pro-Gly-Pro (*n*=12)	Pro-Gly+Gly-Pro (*n*=11)
Plasmin and PA contents, mm²	7.2±0.2	52.9±14.8*	32.0±10.9*	120.0±30.6*
Plasmin content, mm²	0.86±0.10	3.9±1.1*	5.3±1.4**	4.2±1.5**
PA content, mm²	8.6±0.2	52.5±15.8*	26.5±6.8**	94.2±24.5*
Antiplasmin activity, arb. units	16.1±0.5	6.4±1.1*	12.7±0.8*	12.9±1.2*

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

duced the most pronounced increase (by 16.6 times compared to the control) in the total FA, including plasmin and PA activities. This effect was related to a sharp increase in tissue PA activity in these rats. Semax 7.3-fold increased the total FA, which was also associated with an increase in PA activity. Tripeptide Pro-Gly-Pro produced less pronounced effects on fibrinolytic parameters: the total FA increased only by 4.4 times compared to the control. All peptides enhanced plasmin activity to the same extent.

The effects of peptides on PA activity decreased in the following order: Pro-Gly+Gly-Pro>Semax>tripeptide Pro-Gly-Pro.

Semax reduced plasma concentration of antiplasmins by 54.3%, whereas Pro-Gly-Pro and Pro-Gly+Gly-Pro mixture decreased this parameter only by 20%.

Test peptides had practically no effect on plasma fibrinogen concentration (344.0±25.6 mg/100 ml in the control).

It should be emphasized that the test peptides activated the fibrinolytic system by modulating various parameters. All peptides activated tissue PA and reduced plasma concentration of antiplasmins compared to the control.

The antithrombotic activity of Semax and tripeptide Pro-Gly-Pro was demonstrated in experiments with 1.5-h jugular vein clamping. In rats treated with these peptides the weight of thrombi in the clamped vessel was lower by 65% and 81%, respectively (p≤0.001), compared to the control (1.45±0.13 g).

Thus, Semax and tripeptide Pro-Gly-Pro possess pronounced antithrombotic activity.

Our findings suggest that Semax is a more potent anticoagulant than Pro-Gly-Pro and Pro-Gly+Gly-Pro mixture. Moreover, Semax possesses a higher fibrinolytic activity than tripeptide Pro-Gly-Pro. The Pro-Gly+Gly-Pro mixture produces a more pronounced fibrinolytic effect compared to other test peptides. The tripeptide Pro-Gly-Pro exhibits more potent *in vivo* antithrombotic activity than Semax. Thus, these peptides increase the anticoagulant potential of the blood and can be used for the therapy and prevention of thromboses.

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