Effect of Elementary Proline-Containing Peptides on Functional Activity of Anticoagulation System and Primary Homeostasis

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Elementary proline-containing peptides being added to rat platelet-rich plasma or platelet suspension in concentrations of 10^{-1}-10^{-7} mg/ml elicit a considerable antiplatelet effect. Efficiency of inhibition of platelet aggregation increases in the following order: Pro-Gly>Pro-Gly-Pro>Gly-Pro-Gly-Gly. Intravenous injection of these peptides activates blood anticoagulation system in experimental animals.

Key Words: *platelet aggregation; elementary proline-containing peptides*

There are experimental data on various modulating effects of natural and synthetic peptides on blood coagulation system, in particular on initial hemostasis [1,2,5]. In *in vitro* experiments some peptides (thymosine-α1 fragments, thymic peptide fraction, etc.) inhibits platelet aggregation (PA) in platelet-rich plasma (PRP) and platelet suspension induced by various aggregants [7,11]. Proline residue attached to RGD peptide enhances its antiplatelet activity [10].

Some short proline-containing peptides are degradation products of connective tissue proteins (collagen and elastin). Collagen molecule contains numerous Pro-Gly, Gly-Pro, and Pro-Gly-Pro motifs. It has been shown that Pro-Gly and Gly-Pro dipeptides and Pro-Gly-Pro tripeptides activate neutrophils [12,13], possess antiulcer activity, and probably exhibit other regulatory effects.

Our previous experiments have demonstrated that Thr-Lys-Pro-Arg (tuftsin), Pro-Gly, and Trp-Pro inhibit the terminal stage of fibrin formation and enhance lysis of unstable fibrin. Apart from the above effects, intravenously injected Pro-Gly peptide inhibits PA in rats [2]. Native collagen is a well known inductor of AT. It can be hypothesized that dipeptides, products of collagen degradation, exhibit antiplatelet effect due to blockade of collagen-binding sites.

In the present study we explored the effect of elementary proline-containing peptides on PA *in vitro* and *in vivo*.

MATERIALS AND METHODS

Proline-containing peptides used were: Pro-Gly, Pro-Gly-Pro (Institute of Molecular Genetics, Russian Academy of Sciences) and Gly-Pro-Gly-Gly (Sigma). Tuftsin was used in some experiments to compare experimental data with our previous results.

Peptide solutions (10^{-1}-10^{-7}) were added to rat PRP. Blood was drawn from *v.jugularis* of healthy rats with a syringe containing 3.8% sodium citrate (blood:anticoagulant ratio was 9:1) and centrifuged at 100*g* for 5 min. The resultant PRP (0.2 ml) was transferred to plastic cuvettes and was incubated at 37°C for 10 min in the presence of test peptide (0.05 ml), after which PA was measured. Some *in vitro* experiments were performed with isolated and washed platelets suspended in 0.85% NaCl. PA was measured by the increment of light scattering using an aggregometer (Moscow State University). Aggrega-

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TABLE 1. Effect of Proline-Containing Peptides on PA in Rat PRP (Induction with 0.5-1.0 mM ADP, *M±m*)

Sample	PA (%) after addition of peptides in concentrations, mg/ml			
	10^{-1}	10^{-3}	10^{-5}	10^{-7}
0.85% NaCl (control)	100±3.9	100±3.5	100±3.6	100±4.1
Pro-Gly	22.0±4.5**	43.2±5.8**	51.0±7.8**	17.0±5.6**
Pro-Gly-Pro	15.5±5.8**	62.0±4.5*	32.0±3.8**	32.5±4.5**
Gly-Pro-Gly-Gly	77.9±7.5	63.2±1.9*	67.8±1.7*	89.3±2.0
Thr-Lys-Pro-Arg	39.0±4.6**	32.0±0.3**	35.2±0.3**	40.0±0.4**

Note. Here and in Tables 2 and 3: *$p<0.01$, **$p<0.001$ compared with the control.

tion was induced by ADP (Reanal 0.5-1.0 mM final concentration) or thrombin (0.5 U/ml) added to the cuvette at a 1:10 ratio.

In vivo experiments were carried out on 66 male albino rats (Kryukovo nursery) weighing 200-250 g. Experimental rats received intravenous injection (through *v. jugularis*) of test peptides in a concentration of 250 μg/kg (0.5 ml/200 g body weight). Control rats received 0.85% NaCl. Ten minutes later blood was drawn from *v. jugularis* and after 12-min centrifugation at 450*g* the anticoagulation activity of test peptides was evaluated by measuring the routine recalcification and thrombin times, total and nonenzymatic fibrinolytic activities [4], plasminogen activator activity [8], and PA [9].

The data were processed statistically using the Student t test.

RESULTS

Being added to rat PRP in a concentration of 0.1 mg/ml, Pro-Gly and Pro-Gly-Pro reduced PA to 48 and 15%, respectively. Marked reduction occurred only when the sample was incubated at 37°C for 10 min in the presence of test peptide.

As seen from Table 1, maximum inhibition of PA was attained with Pro-Gly, Pro-Gly-Pro, and tuftsin. It is remarkable that peptides were highly effective even in low concentrations (inhibition to 17-32% at a concentration of 10^{-7} mg/ml). Intermediate concentrations (10^{-3}-10^{-5} mg/ml) were apparently less effective (inhibition to 32-62%), but this tendency requires additional verification.

Inhibition of PA was observed not only in PRP but also in suspension of washed rat platelets (Table 2). For instance, Pro-Gly in concentrations of 10^{-5}-10^{-7} mg/ml inhibited PA to 50 and 59%, respectively. Tripeptide Pro-Gly-Pro in concentrations of 10^{-3} and 10^{-5} mg/ml inhibited aggregation of washed platelets to 49 and 35%, respectively. The same peptide without preincubation exerted a less pronounced effect (72%).

In the next experimental series peptides were injected intravenously. Ten minutes after intravenous injection of 250 μg/kg Pro-Gly we observed a significant increase in blood anticoagulation activity, total and nonenzymatic fibrinolysis, and plasminogen activator activity and a marked decrease in PA (Table 3) attesting to a considerable activation of blood anticoagulation system [3] and marked suppression of initial hemostasis. Injection of the same dose of Pro-Gly-Pro activated only fibrinolytic part of the anticoagulation system. The dose of 1.5 mg/kg induced a considerable increase in anticoagulation and fibrinolytic activities and a marked inhibition of PA (Table 3).

Gly-Pro-Gly-Gly in a dose of 250 μg/kg had little effect on PA (88% of the control level) but considerably activated fibrinolytic component of the anticoagulation system (plasminogen activator and total fibrinolytic activities increased by 47 and 31%, respectively).

Our experiments showed that elementary proline-containing peptides can activate the anticoagulation system; they not only enhance blood fibrinolytic and anticoagulation capacity, but exhibit antiplatelet activity. The involvement of these peptides, products of collagen and elastin degradation, into regulation of blood coagulation and fibrinolysis was demonstrated. Unlike native collagen, a well-known inductor of aggregation, elementary proline-containing peptides can act as effective inhibitors of PA.

TABLE 2. Effect of Proline-Containing Peptides on Aggregation of Washed Rat Platelets (Induction with ADP, *M±m*)

Sample	PA (%) after addition of peptides in concentrations, mg/ml		
	10^{-3}	10^{-5}	10^{-7}
0.85% NaCl (control)	100±3.45	100±4.0	100±3.45
Pro-Gly		50.1±5.9*	59.2±6.1*
Pro-Gly-Pro	49.4±3.8*	35.3±4.6*	

TABLE 3. Hemostatic Parameters in Rats after Single Intravenous Injection of Pro-Gly and Pro-Gly-Pro (*M±m*)

Group	Recalcification time, sec	Fibrinolysis, mm			PA, %
		total	non-enzymatic	plasminogen activator	
0.85% NaCl (control)	141±11.9	39.5±2.3	18.8±0.5	5.1±0.9	100±12.2
Pro-Gly, 250 μg/kg	493±42*	50±3.6*	23±2.3	7±0.9	51±10*
Pro-Gly-Pro, 1.5 mg/kg	225±14.7*	65.6±4.1*	35.8±3.0*	29±5.3*	13.2±6.8*

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