PHYSIOLOGY

Prevention of Thrombus Formation with Glyprolines on Various Models of Prethrombotic State and Thrombosis in Rats

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Peroral administration of peptide Pro-Gly-Pro to 10-11-month-old rats with modeled prethrombotic state normalized functions of the anticoagulation system and produced a potent antiplatelet effect. Peroral administration of Pro-Gly peptide before provocation of thrombin generation and thrombus formation prevented death of animals from thrombosis. Experiments on rats with venous thrombosis induced by stasis and administration of thrombin showed that pretreatment with Pro-Gly peptide decreased the weight of formed thrombi.

Key Words: *glyprolines; antithrombotic and antiplatelet properties of peptides; prethrombotic state; thrombosis*

Our previous *in vivo* and *in vitro* experiments demonstrated pronounced anticoagulant activity of glyprolines Pro-Gly, cPro-Gly, Pro-Gly-Pro, and Gly-Pro-Gly-Gly. *In vitro* and *in vivo* studies on healthy animals revealed anticoagulant, antiplatelet, fibrin-depolymerizing, and fibrinolytic effects of glyprolines [1,2]. The test peptides in a concentration of 10^{-2}-10^{-9} M *in vitro* inhibited aggregation in platelet-rich plasma induced by aggregation agonists ADP, thrombin, and collagen [8]. Intravenous, peroral, intranasal, and intraperitoneal administration of peptides to animals increased blood anticoagulant activity, depolymerized non-stabilized fibrin (NF), promoted fibrinolysis, activated tissue plasminogen activator, and inhibited fibrin stabilization and platelet aggregation (PA) [3,9,13].

The direct antithrombotic effect produced by peptides that enter the composition of natural proteins (collagen and elastin) is of considerable interest.

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Here we studied the antithrombotic effect of peptides Pro-Gly and Pro-Gly-Pro on various models of prethrombotic state and thrombosis in rats.

MATERIALS AND METHODS

We used pure peptides Pro-Gly and Pro-Gly-Pro synthesized at the Institute of Molecular Genetics (Russian Academy of Sciences).

Prethrombotic state and thrombosis were modeled in 102 male outbred albino rats. Prethrombotic state was induced in 10-11-month old animals (n=37, body weight 350-400 g) demonstrating age-related suppression of the anticoagulation system verified as described previously [4]. Some rats perorally received Pro-Gly-Pro in a daily dose of 1 mg/kg (0.5 ml) for 7 days. Control animals received an equivalent volume of 0.85% NaCl. Antithrombotic activity of the peptide was determined in blood tests.

Model I of thrombosis was studied in 33 rats weighing 200-220 g. Homologous tissue thromboplastin from rat brain (coagulant activity 18-20 sec) was injected in a single dose of 0.8 ml into the jugular vein

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[7]. Administration of the thromboplastic agent inducing endogenous thrombin generation in the blood was followed by thrombus formation, respiratory dysfunction, and motor disturbances. The number of dead animals with thrombi in the heart, lungs, liver, kidneys, and other organs was determined 3-4 h after thromboplastin administration. The rats of 2 experimental groups with thrombosis perorally received peptides Pro-Gly and Pro-Gly-Pro in doses of 125 μg/kg and 1 mg/kg for 4 days at 24-h intervals before treatment with thromboplastin. The number of animals died from thrombosis was determined 3-4 h after thromboplastin administration. The antithrombotic effect of the peptides was evaluated (compared to the control).

Model II of thrombosis and prethrombotic state was investigated in 32 healthy rats by the method of Wessler [15]. The dipeptide Pro-Gly was injected intraperitoneally in a dose of 0.5 mg/kg for 5 days at 24-h intervals before thrombus formation. Thrombosis was induced in a jugular vein segment isolated with forceps. After 1 h the segment was dissected, and the thrombus was removed, treated, and weighted. We compared the weights of thrombi in control and experimental animals. The decrease in the weight of thrombi characterized the antithrombotic effect of peptides.

The efficiency of the test peptides was determined by functional activity of the anticoagulation system. Plasma and platelet hemostasis was determined by total fibrinolytic activity (TFA), depolymerization of NF [6], activity of tissue plasminogen activator (APA), partial thromboplastin time (PTT) [5], and ADP-induced PA in platelet-rich plasma (evaluated by increment of light scattering on an aggregometer constructed at Moscow State University) [11]. The blood was taken from the jugular vein using a syringe with the anticoagulant sodium citrate (anticoagulant/blood ratio 1:9). Platelet-rich and platelet-depleted plasma samples were obtained under different centrifugation conditions. The results were analyzed by Student—Fischer test.

RESULTS

Peroral administration of the peptide Pro-Gly-Pro for 7 days at 24-h intervals activated the anticoagulation system and normalized antithrombotic activity in 10-11-month-old rats with impaired anticoagulant functions and prethrombotic state.

Inhibition of fibrinolysis, reduction of APA, and stimulation of PA were observed in group 2 control rats aging 10-11 months (compared to young animals of group 1, Table 1). Chronic administration of the tripeptide in a dose of 1 mg/kg increased functional activity of the anticoagulation system in 10-11-month-old rats (group 3). In these animals parameters of the anticoagulation system returned to a level observed in young rats. TFA, NF, and APA increased by 1.85, 1.80, and 4 times, respectively. Pro-Gly-Pro produced a strong antithrombotic effect. The intensity of PA decreased by 2.7 times compared to that in control old rats of group 2 not treated with the peptide. These data indicate that the tripeptide Pro-Gly-Pro produced antiplatelet and antithrombotic effects.

We studied the antithrombotic effect of peptides Pro-Gly and Pro-Gly-Pro in rats with thrombosis produced by intravenous injection of tissue thromboplastin (Table 2). The thromboplastic agent induced generation of the endogenous coagulant enzyme thrombin [7]. Some control animals receiving sodium chloride died from thrombosis 3-4 h after thromboplastin administration. Autopsy revealed thrombi in heart ventricles and auricle, pulmonary, hepatic, and renal vessels. Experimental rats perorally received Pro-Gly (series I) and Pro-Gly-Pro (series II) in doses of 25 and 200 μg/200 g, respectively, for 4 days at 24-h intervals. Thromboplastin was injected 1-1.5 h after the last infusion of the peptides. After 3-4 h the number of animals receiving peptides and died from thrombosis was 20-24% lower than in the control. These rats were characterized by less pronounced motor dysfunction and more rapid recovery of voluntary movements.

TABLE 1. Parameters of Hemostasis in Animals with Prethrombotic State after Chronic Peroral Administration of Pro-Gly-Pro in a Dose of 1 mg/kg (*M*±*m*)

Parameter	Group		
	1 (3.5-month-old control animals)	2 (10-11-month-old control animals)	3 (experiment)
TFA, mm^2	38.4±2.3	28.0±2.1**	52.2±1.7*
NF, mm^2	22.0±1.1	17.6±1.9**	32.5±1.9**
APA, mm^2	12.7±0.7	9.1±0.7**	34.7±3.1*
PTT, sec	66.4±2.6	65.0±2.3	99.0±4.2*
PA, %	100.0±5.1	145.0±4.8*	40.0±2.4*

Note. *$p<0.01$ and **$p<0.05$ compared to group 1.

TABLE 2. Survival of Animals Perorally Receiving Pro-Gly and Pro-Gly-Pro for 4 Days before Intravenous Injection of Tissue Thromboplastin

Group	Number of animals		
	initial	died	survived, %
Series I			
sodium chloride and thromboplastin (control)	12	4	67
peptide Pro-Gly and thromboplastin	11	1	91
Series II			
sodium chloride and thromboplastin (control)	5	3	40
peptide Pro-Gly-Pro and thromboplastin	5	2	60

The effect of Pro-Gly on thrombus formation was studied by the method of Wessler. Intraperitoneal administration of Pro-Gly in a dose of 0.5 mg/kg at 24-h intervals (5 injections) before the experiment decelerated thrombus formation in the jugular vein and decreased the weight of thrombi by 40% compared to the control (1.37±0.20 and 2.25±0.15 g, respectively). The decrease in the weight of thrombi after treatment with peptides was accompanied by suppression of PA and activation of fibrinolysis. The reduced weight of thrombi in experimental animals reflected antithrombotic activity of Pro-Gly.

Our results indicate that glyprolines Pro-Gly and Pro-Gly-Pro produce an antithrombotic effect on various models of thrombosis and prethrombotic state in animals. The antiplatelet effect of peptides and other compounds is the major constituent of their antithrombotic activity. Our *in vitro* and *in vivo* studies showed that peptides Pro-Gly and Pro-Gly-Pro administered *in vitro* and *in vivo* produced antiplatelet effects [2,9, 10]. The present work was performed on 10-11-month-old rats with suppressed functional activity of the anticoagulation system and impaired plasma and platelet homeostasis. Chronic peroral administration of Pro-Gly normalized functions and activated the anticoagulation system, decreased the intensity of PA to a normal level, and prevented thrombosis after provocation.

Peroral administration of Pro-Gly and Pro-Gly-Pro produced an antithrombotic effect in rats with thrombosis induced by intravenous injection of tissue thromboplastin. Pretreatment with peptides Pro-Gly and Pro-Gly-Pro before injection of thromboplastin decreased the mortality rate from thrombosis. These data illustrate the antithrombin and antithrombotic effects of the test peptides. Our previous experiments revealed antiplatelet activity of the studied peptides [3,8]. Treatment with peptides before thromboplastin administration inhibits PA and prevents thrombus formation in the blood. Experiments on rats with thrombosis and prethrombotic state revealed an antithrombotic effect of intraperitoneal pretreatment with Pro-Gly.

Several peptide compounds with high molecular weight were tested as medicinal preparations in foreign hospitals for patients with cardiovascular diseases over the last 10 years. The disulfide-bound cyclic heptapeptide Eptifibatide from venom of *Sistutis m. Barbouri* snakes and peptide preparation Tirofiban with the amino acid sequence RGD act as specific inhibitors of glyproline IIb/IIIa receptors on the platelet membrane and inhibit ADP-induced PA. Infusion of these preparations to patients with ischemia of the heart and brain, angina pectoris, and postinfarction disorders inhibits PA by 80-97% and inactivates blood thrombin [12]. Large-scale clinical trials involving more than 10,000 patients demonstrated cardioprotective activity of these preparations and decrease in mortality rate. Sometimes they produced side effects. For example, administration of peptides (particularly in combination with acetylsalicylic acid or heparin) increased the time of bleeding [14].

Experiments on animals with thrombosis and prethrombotic state indicate that antithrombotic activity of peptides Pro-Gly and Pro-Gly-Pro should be studied in formalized preclinical trials.

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