Arginine-Containing Tripeptide Pro-Arg-Gly Is Involved in the Regulation of the Function of Anticoagulation and Insular Systems under Persistent Hyperglycemia

L. A. Andreeva, N. F. Myasoedov, T. A. Shubina*, M. E. Grigorieva*, and L. A. Ljapina*

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Intranasal administration of regulatory peptide PRG to rats against the background of persistent hyperglycemia improves blood antiplatelet and anticoagulant-fibrinolytic potential and normalizes blood sugar in comparison with the corresponding parameters in control animals that did not receive the peptide. The fibrinolytic and antiplatelet activities of blood plasma remained elevated 6 days after peptide withdrawal against the background of unchanged glucose administration.

Key Words: *peptides; hyperglycemia; hemostasis system*

A correlation was found between elevated blood sugar and impaired function of the anticoagulation system and, consequently, enhanced blood clotting in rats during the development of insulin-dependent diabetes mellitus [7]. Glycemia is associated with increased platelet aggregation [8] and decreases anticoagulation activity and enzymatic and non-enzymatic fibrinolytic properties of the blood [2]. Some studies have shown that proline-containing regulatory peptides Pro-Gly, Pro-Gly-Pro, and Gly-Pro produce antiplatelet, anticoagulant, fibrin-depolymerizing, and antifibrin-stabilizing effects on the blood [1,6]; they can protect the insular system and prevent the development of experimental type 1 diabetes mellitus [2,9]. Glyprolines modified by binding of arginine in different positions of their molecules received much recent attention.

Arginine is a donor and a carrier of nitrogen in the body. It improves the rheological properties of the blood and prevents platelet aggregation thus reducing the risk of thrombosis and atherosclerotic plaques and contributing to the neutralization of free radicals that are generated in diabetes mellitus [11]. The positive effect of L-arginine in type 2 diabetes mellitus was noted [10,15]. It regulates the normal secretion of insulin in the pancreas and increases the sensitivity of peripheral insulin receptors, thus reducing the risk of diabetes [12-14]. We have previously studied glyprolines carrying N-terminal or C-terminal arginine and demonstrated their protective effect on the function of the anticoagulant and insular systems during the development of type 1 or type 2 experimental diabetes. However, the effects of glyprolines carrying arginine at non-terminal position were not described.

Here we studied antiplatelet and antidiabetic activities of Pro-Arg-Gly (PRG) peptide, an arginine-containing glyproline administered intranasally to rats with persistent experimental hyperglycemia, similar to that in type 2 diabetes mellitus and accompanied by impaired function of the anticoagulation system.

MATERIALS AND METHODS

We used PGP peptide synthesized at the Institute for Molecular Genetics, Russian Academy of Sciences. The experiments were carried out on 5-6-months-old

Institute of Molecular Genetics, Russian Academy of Science, Moscow; *Department of Human and Animal Physiology, Biological Faculty, M. V. Lomonosov Moscow State University, Russia. ***Address for correspondence:*** lyapinal@mail.ru. L. A. Ljapina

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outbred albino male rats (n=52) weighing 180-220 g and feeding a standard laboratory diet.

Persistent hyperglycemia (type 2 diabetes mellitus) was induced in animals by repeated intragastric administration of 40% glucose solution in a dose of 0.5 ml per 200 g body weight (glucose challenge).

The experiment was carried out as follows: the animals were given glucose for 14 days. Starting from day 12, protamine sulfate solution was injected intramuscularly (0.2 ml/200 mg per 200 g body weight for 3 days) and PRG peptide solution was administered intranasally (0.05 ml/200 μg per 200 g body weight; 2 times a day on days 12 and 13 days and once on day 14). The control animals received glucose and equivalent volume of 0.85% NaCl instead of the peptide. On the day of the experiment (day 14), the rats received glucose and protamine sulfate and after 30 minutes the peptide (experimental group) or 0.85% NaCl (control group) were administered. The blood for analysis was taken 1 h after the last dose of the peptide in the experimental group or 0.85% NaCl in the control group and then 6 days after peptide (or saline) withdrawal against the background of glucose administration. Group "normal" consisted of Healthy rats that received no substances were additionally used (normal).

The blood (2 ml) was sampled from *v. jugularis* using 3.8% sodium citrate as the anticoagulant (9:1 blood-citrate ratio).

The following biochemical parameters of the blood plasma were evaluated: total fibrinolytic activity (TFA) and non-enzymatic fibrinolytic activity (NFA) on non-stabilized fibrin in our modification [7], plasminogen tissue activator activity (PAA), enzymatic fibrinolytic activity (EFA) in the euglobulin fraction of the plasma [3], and activated partial thromboplastin time (APTT) [5]. Levels of Hageman factor (XIIa)-dependent fibrinolysis (HFDF) were determined using a commercial test system (NPO Renam). In platelet-rich plasma, platelet aggregation was assessed by the method [4] on an aggregometer manufactured at the Moscow State University using 2-5 μM ADP solution as the aggregation inductor. The blood sugar level was routinely measured on an Accutrend GC analyzer using test strips (Roche). Blood samples were centrifuged at 1000g for 5 min for evaluation of platelet aggregation (isolation of platelet-rich plasma) and at 3000g for10-12 min for coagulation tests and blood sugar measurement in the platelet-poor plasma.

The results were statistically processed using Student's t test.

RESULTS

Experiments showed that TFA and NFA in animals receiving PRG peptide increased by 32 and 69%, respectively, in comparison with the control; plasma PAA increased by 104.8%, EFA in the euglobulin fraction by 58%, and HFDF by 92% in comparison with the corresponding parameters in controls. Platelet aggregation in the experimental group decreased to 43.8%. The blood sugar level in experimental rats was lower than in controls by 3.4 mmol/liter and below the initial level by 0.6 mmol/liter. APTT reflecting anticoagulation activity of the plasma in the presence of the peptide exceeded the control level by 93% (Table 1).

In 6 days after peptide and protamine sulfate withdrawal, PAA, EFA in the euglobulin fraction, and plasma HFDF in experimental rats still surpassed the control values by 24, 26, and 110%, respectively; platelet aggregation remained at the level of 42.8%, anticoagulant activity surpassed the control values by only 12%. Hence, elevated fibrinolytic and antiplatelet activities of the blood plasma were observed over 6 days after cessation of peptide administration (Table 2).

During this period, blood glucose in controls slightly increased by 0.5 mmol/liter after drug withdrawal in comparison with that in experimental rats (Table 2).

When analyzing the above data it should be noted that blood sugar level in experimental rats was reduced after 5-fold administration of PRG peptide during the development of type 2 diabetes mellitus (against the background of sugar load), whereas hyperglycemia and the development of type 2 diabetes mellitus were found in the absence of the peptide (saline administration). Hemostasis parameters such as anticoagulant activity, different types of fibrinolytic activity, and platelet aggregation indicated hypocoagulation against the background of introduced peptide in the experimental group. In contrast, increased blood clotting was observed in the control group. Blood glucose levels in experimental and control animals almost returned to normal 6 days after cessation of peptide administration. At the same time, some hemostasis parameters in experimental group were significantly more than control values for fibrinolysis (TFA, NFA, HFDF) and platelet aggregation indicating reduced blood clotting (Table 2).

Hence, 5-fold administration of PRG peptide at against the background of sugar load produced anticoagulant and fibrinolytic effects mediated by enzymatic and non-enzymatic mechanisms, reduces platelet aggregation, and contributes to normalization of blood sugar levels. Our results indicate that not only L-arginine [10,15], but also PRG, a glyproline carrying arginine in the non-terminal position, prevents the development of type 2 diabetes mellitus under conditions of excessive glucose intake. In parallel, the analyzed peptide, judging from coagulation parameters, prevented the suppression of anticoagulation system occurring in diabetes.

TABLE 1. Blood Parameters after Daily Intranasal Administration of PRG Peptide against the Background of Glucose Load and Protamine Sulfate Treatment (*M*±*m*)

Blood parameters	Controls	Experiment	Normal
Sugar level, mmol/liter	7.5±0.7^{++}	4.1±0.1**	4.7±1.7
APTT, sec	17.6±3.1	34.0±3.7**	21.7±1.3
TFA, mm^2	26.7±1.7^{++}	45.2±2.3**	36.7±0.9
NFA, mm^2	19.2±1.1^{+}	32.2±1.8**	23.2±0.4
Index of platelet aggregation	5.8±1.5	2.5±0.8*	8.45±2.20
HFDF, min	8.1±0.9^{++}	4.2±0.5**	3.0±0.1
PAA, mm^2*	18.8±6.3	38.5±7.4*	26.0±6.8
EFA, mm^2	77.8±4.9	124.5±10.4**	81.0±0.1

Note. Here and in Table 2: *p<0.05, **p<0.01 in comparison with controls; ^{+}p<0.05, ^{++}p<0.01 in comparison with normal.

TABLE 2. Hemostasis Parameters and Blood Sugar Level 6 days after Cessation of PRG Peptide (Experiment) or 0.85% NaCl Solution (Control) Treatmeent against the Background of Intragastric Glucose Administration

Blood parameters	Controls	Experiment	Normal
Sugar level, mmol/liter	5.3±1.2	4.9±0.7*	6.6±0.6
APTT, sec	23.0±1.3	26.8±1.3*	24.2±1.2
TFA, mm^2	28.5±0.9^{+}	36.7±2.1**	32.6±0.9
NFA, mm^2	18.8±1.1	23.2±0.9**	20.8±0.9
Index of platelet aggregation	12.6±1.9	5.4±1.6**	14.4±3.4
HFDF, min	7.9±0.9^{++}	3.7±0.5**	4.9±0.5
PAA, mm^2	18.0±3.8^{++}	22.4±4.2*	36.0±6.8
EFA, mm^2	83.5±6.8^{++}	105.1±9.5**	99.0±0.1

Thus, 5-fold intranasal administration of regulatory peptide PRG to rats with persistent hyperglycemia improves antiplatelet and anticoagulant-fibrinolytic potential of the blood and normalizes blood sugar in comparison with the corresponding values in control animals not treated with the peptide. The fibrinolytic and antiplatelet background of blood plasma remained elevated over 6 days after peptide withdrawal against the background of unchanged glucose supply. The test regulatory peptide Pro-Arg-Gly produced a protective antithrombotic and antidiabetic effect under conditions of persistent hyperglycemia caused by intragastric administration of glucose in high concentrations.

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