Anticoagulant, Fibrinolytic, and Hypoglycemic Effects of Tetrapeptide Arg-Pro-Gly-Pro

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It was found that 3-fold intranasal administration of Arg-Pro-Gly-Pro peptide in a dose of 1 mg/kg body weight under conditions of experimental persistent hyperglycemia prevents the development of diabetes in experimental rats and produces normoglycemic, anticoagulant, fibrinolytic, and antiplatelet effects.

Key Words: *diabetes; hemostasis; peptides*

We have previously shown that glyprolines Pro-Gly, Pro-Gly-Pro, Gly-Pro exhibit anticoagulant, fibrin-depolymerizing, anti-fibrin-stabilizing [1,5] and antiplatelet [8] activities. It was found that regulatory peptides Pro-Gly-Pro, Pro-Gly Pro-Gly-Pro-Arg, Pro-Gly-Arg protect normal function of the insular system in experimental animals with alloxan-induced insulin-dependent diabetes mellitus [2,6,10] accompanied by anticoagulation system dysfunction [9]. Platelet aggregation in the plasma increases [8], whereas anticoagulant activity and enzymatic and non-enzymatic fibrinolytic activities decrease under these conditions [2].

Arginine present in these peptides is known to stimulate the production of NO, which promotes antithrombotic activity by reducing blood viscosity and platelet aggregation. Arginine also neutralizes free radicals generated in some pathologies, *e.g.* atherosclerosis and diabetes [12]. L-arginine administered to patients with type 2 diabetes mellitus significantly increases insulin receptor sensitivity in the liver and peripheral tissues [14]. Experiments on rats showed that administration of L-arginine to animals with experimental diabetes mellitus reduces polydipsia and normalizes plasma protein concentration. According to published reports, L-arginine potentiates the increase in peripheral insulin receptor sensitivity and growth of capillaries in skeletal muscle [12] and inhibits hemoglobin glycosylation and LPO in diabetes mellitus, thus reducing the risk of complications [13]. Lack of arginine increases the risk of type 2 diabetes mellitus, as insulin-sensitive tissues become resistant to insulin [11].

Here we studied anticoagulant and antidiabetic activities of peptide Arg-Pro-Gly-Pro (ArgPGP) in experimental sustained hyperglycemia developing in type 2 diabetes mellitus and accompanied by anticoagulation system dysfunction.

MATERIALS AND METHODS

The peptide ArgPGP was synthesized at the Institute for Molecular Genetics, Russian Academy of Sciences.

The experiments were carried out on 10-months-old white outbred male rats (n=48) weighing 270-350g and kept on a standard laboratory diet.

Persistent hyperglycemia in animals was caused by repeated (3-8 days) intragastric administration of 40% glucose solution in a dose of 0.5 ml per 200 g body weight (glucose challenge).

Two series of experiments were performed. In series I, experimental animals received prophylactic intranasal administration of peptide ArgPGP (0.05 ml/200 μg per 200 g body weight) every 24 h for 4 days and then (days 5-7) the peptide was injected in this dose and simultaneously glucose challenge was

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performed. The control group received intranasally the same volume of 0.85% NaCl. The blood was sampled 1 h after the last administration of preparations.

In series II, the experimental and control animals received standart glucose load for 1 week and then (starting from day 8) intranasally received ArgPGP peptide (experimental group) or 0.85% NaCl (controls) in the above-specified doses. The blood was sampled in 30-40 min after peptide administration for measuring glucose content. The peptide was administered intranasally 3 times for 2 days simultaneously with glucose challenge and the blood for biochemical tests was taken 1 h after the last administration of the peptide (experiment) or 0.85% NaCl (control) and then in 5 days after the last peptide dose (only for measuring of glucose concentration). The rats in the "normal" group received no substances.

The blood (2 ml) was sampled from the jugular vein; sodium citrate (3.8%) was used as preservative (9:1).

Blood samples were centrifuged in two different regimens: 5 min at 1000*g* for platelet aggregation test (isolation of platelet-rich plasma) or 10-12 min at 3000*g* for coagulation tests and for measuring glucose concentration in platelet-poor plasma.

The following biochemical parameters of blood plasma were determined: total fibrinolytic activity (TFA), non-enzymatic fibrinolytic activity (NFA), activity of tissue plasminogen activator (tPA) [7], and activated partial thromboplastin time (APTT) [4]. Platelet aggregation was determined by the method [3] using the aggregometer manufactured at the Moscow State University. Coagulogram was recorded by N-334 coagulometer (Russia). The following parameters were calculated: T_1, the starting of clot formation; T_2, the end of clot formation; T, clotting time. The blood sugar level was defined using the modified Hagedorn–Jensen method [7]. The results were analyzed statistically using Students *t* test.

RESULTS

In series I, the test peptide significantly increased blood TFA and tPA in experimental animals: these parameters 1.5- and 1.4-fold surpassed the control values. NFA and platelet aggregation parameters were similar in the experimental and control groups. However, APTT values in the experimental group tended to decrease in comparison with the control (Table 1).

Blood sugar in experimental rats was significantly (by 1.5 times) below the control.

Since series I, sustained hyperglycemia was accompanied by a sharp increase in platelet aggregation (by more than 100%) in both experimental and control animals, some changes in the experimental protocol were needed to reveal the anticoagulant action of the peptide under these conditions.

In series II, activation of the anticoagulant function was demonstrated in 1 h after 3-fold intranasal administration of the test peptide against the background of hyperglycemia (Table 2). Thus, under constant supply of blood sugar, TFA in the plasma of experimental animals significantly increased by 1.2 times, NE by 1.4 times, tPA by 2.9 times, and APTT by 1.2 times. Platelet aggregation significantly decreased by 25%, and coagulogram parameters T_1 and T_2 and increased by 2.3 and 1.3 times, respectively, in comparison with the corresponding control values. Therefore, this administration route was most effective for manifestation of anticoagulant, fibrinolytic, and anti-aggregation activities of the peptide in rat plasma.

It was found that experimental conditions of series II were most favorable for the hypoglycemic action of ArgPGP peptide.

Blood sugar 1 h after single administration of the peptide under conditions of glucose challenge test increased by 34.8 mg% in experimental rats and by 22.4 mg% in the control group (in comparison with

TABLE 1. Hemostasis Parameters and Glucose Level in Rats Receiving Prophylactic Intranasal Administration ArgPGP in a Dose of 1 mg/kg (Series I; *M*±*m*)

Experimental condition	TFA, mm²	NFA, mm²	tPA, mm²	APTT, sec	Platelet aggregation, %	Glucose level, mg%
Control group: 0.85% NaCl+glucose (*n*=6)	26.4±2.1	20.0±1.3	28.8±3.1	32.8±2.5	215.0±4.5⁺	151.6±10.7
Experiment: ArgPGP+glucose (*n*=6)	38.3±2.2*	23.8±1.7	40.0±3.6*	24.7±1.9	228.0±6.5⁺	98±7.3*
Normal (*n*=4)	34±1.4	24.6±1.0	33.3±1.7	31.7±1.9	100±2	68.0±5.5

Note. Here and in Table 2: $p<0.01$ in comparison with: *control group, ⁺normal group.

TABLE 2. Hemostasis Parameters in Rats Receiving Therapeutic Intranasal Administration of ArgPGP in a Dose of 1 mg/kg against the Background of Persistent Hyperglycemia (Series II; *M*±*m*)

Experimental condition	TFA, mm^2	NFA, mm^2	tPA, mm^2	APTT, sec	Platelet aggregation, %	T_1, sec	T_2, sec	T, sec
Control group: 0.85% NaCl+glucose (*n*=6)	30.2±1.4	20.4±1.5	6.8±0.5	23.8±0.3	150.0±2.7	37.0±9.9	143±29	106.0±21.6
Experiment: ArgPGP+glucose (*n*=6)	38.6±1.5*	28.5±1.3*	20.1±0.4*	28.±0.6*	75.0±3.5*	76.0±12.0*	193±16*	116±21
Normal (*n*=4)	39.3±5.0	30.6±2.2	24.0±1.8	29.6±6.1	100	45	185.0±3.7*	142.0±3.5

TABLE 3. Blood Glucose Levels in Rats at Different Terms after Intranasal Administration of Peptide ArgPGP under Condition of Persistent Hyperglycemia (Series II; *M*±*m*)

Experimental condition	Blood glucose level in rats, mg%		
	1 h after single administration of the peptide against the background of glucose challenge	1 h after 3-fold administration of the peptide against the background of glucose challenge	5 days the last peptide administration
Control group: 0.85% NaCl+glucose (*n*=6)	102.4±4.2^{++}	172.0±6.1^{++}	80.8±3.1
Experiment: ArgPGP+glucose (*n*=6)	114.8±6.1^{++}	124.0±4.6*$^{+}$	81.0±23.9
Normal (*n*=4)	80.0±2.7	92.0±5.5	61.3±33.1

Note. *p<0.01 in comparison with the control; ^{+}p<0.05, ^{++}p<0.01 in comparison with the normal.

the "normal" group, Table 3). After 3-fold administration of the peptide, sugar level in the experimental group remained virtually unchanged, but surpassed the normal values by 32 mg% and in control group it significantly surpassed the normal by 80 mg%. Five days after the last ArgPGP dose, a decrease in blood glucose levels to 80-81 mg% was recorded in both groups and these values did not differ significantly from normal.

Thus, 3-fold administration of peptide ArgPGP against the background of persistent hyperglycemia reduced blood glucose level in comparison with the control, while severe hyperglycemia characteristic of type 2 diabetes was observed after administration of saline. APTT, coagulogram parameters, TFA, NFA, tPA activity, and platelet aggregation attested to either hypocoagulation in rats receiving the peptide or increased blood clotting in rats receiving saline.

Hence, ArgPGP peptide produced antocoagulant and fibrinolytic effects under conditions of glucose challenge test, reduced platelet aggregation, and prevented the development of persistent hyperglycemia.

REFERENCES

1. I. P. Ashmarin, A. A. Kamensky, L. A. Lyapina, *et al*., *Vopr. Biol., Med. i Farm. Khimii*, No. 1, 24-27 (2002).
2. I. P. Ashmarin, L. A. Lyapina, L. A. Andreeva, *et al*., *Tromboz, Gemostaz i Reologiya*, No. 2, 38-43 (2008).
3. A. L. Berkovskii, S. A. Vasilev, L. V. Zherdeva, *et al*., *Handbook for the Study of the Platelet Aggregation and Adhesion Activity* [in Russian], Moscow (2003).
4. V. V. Dolgov and P. V. Svirin, *Laboratory Diagnosis of Hemostatic Disorders* [in Russian], Moscow (2005).
5. L. A. Lyapina, V. E. Pastorova., T. J. Obergan, *et al*., *Izv. RAN*, No. 2, 193-203 (2006).
6. L. A. Lyapina, L. A. Andreeva, A. M. Ulyanov, *et al*., *Tromboz, Gemostaz i Reologiya*, No. 2, 53-58 (2011).
7. L. A. Lyapina, V. E. Pastorova., A. M. Ul'yanov. *et al*., *Vestn. MGU, Ser. 16, Biologiya,* No. 1, 7-10 (2002).
8. V. E. Pastorova, L. A. Lyapina, and I. P. Ashmarin, *Byull. Eksp. Biol. Med.,* **138**, No. 10, 364-367 (2003).
9. A. M. Ul'yanov, *Vestn. MGU, Ser. 16, Biologiya,* No. 4, 32-37 (1994).
10. A. M. Ulyanov, L. A. Lyapina, and T. A. Shybina, *Vopr. Biol., Med. i Farm. Khimii*, No. 4, 19-21 (2005).
11. J. Marin and M. A. Rodriguez-Martinez, *J. Auton. Pharmacol*., **15**, No. 4, 279-307 (1995).

12. G. K. McConell, *Curr. Opin. Clin. Nutr. Metab. Care*, **10**, No. 1, 46-51 (2007).
13. J. D. Mendez and F. L. Balderas, *Biomed. Pharmacother*., **60**, No. 1, 26-31 (2006).
14. P. M. Piatti, L. D. Monti, G. Valsecchi, *et. al*., *Diabetes Care*, **24**, No. 5, 875-880 (2001).