



COMPLEX COMPOUND OF PRO-GLY-PRO-LEU WITH HEPARIN: HYPOGLYCEMIC, FIBRINOLITIC AND ANTICOAGULANT EFFECTS IN RATS WITH HYPERGLYCEMIA

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Previously it was shown that the use of regulatory peptides of the glyprolin family helps to normalize the hemostasis system and blood glucose levels in experimental resistant hyperglycemia in rats, similar to type 2 diabetes mellitus in humans. It is also known that the anticoagulant heparin inhibits blood coagulation and exhibits a hypoglycemic effect in the body.

The aim of the study is to obtain a complex of the Pro-Gly-Pro-Leu (PGPL) peptide and the unfractionated heparin, to study its effect on glucose and anticoagulant fibrinolytic properties and show its ability to restore the impaired functions of the insular and coagulating blood systems in experimental hyperglycemia in rats.

Materials and Methods. Laboratory Wistar male rats, intact and with experimentally induced hyperglycemia, were used in the experiment. A complex compound of PGPL and heparin was created with a component ratio of 1:1 (mol/mol), which was administered intranasally to hyperglycemic rats once a day for 5 days at the dose of 1 mg/kg. Similarly, the constituent parts of the complex were administered in equivalent amounts. The anticoagulant activity was determined by the test of activated partial thromboplastin time, fibrinolysis parameters – by tests of total, enzymatic and non-enzymatic fibrinolytic activities, as well as the activity of a tissue plasminogen activator. In addition, blood glucose was measured using special test strips.

Results. The use of the PGPL-heparin complex in the animals with hyperglycemia led to normalization of blood glucose levels, an increase in the anticoagulant and fibrinolytic background of blood plasma. These effects persisted for 6 days after the cancellation of the peptide-heparin complex administration to rat.

Conclusion. In the development of experimental hyperglycemia, the PGPL complex with heparin exhibits a combined hypoglycemic, anticoagulant and fibrinolytic enzymatic and non-enzymatic nature of the effect. In the future, the studied peptide-heparin complex can be used for the prevention and treatment of type 2 diabetes mellitus, complicated by increased blood coagulation.

Key words: anticoagulant activity; blood glucose level; complex compound; fibrinolysis; glyprolin peptides; hyperglycemia; type 2 diabetes mellitus (T2DM)

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КОМПЛЕКСНОЕ СОЕДИНЕНИЕ PRO-GLY-PRO-LEU С ГЕПАРИНОМ: ГИПОГЛИКЕМИЧЕСКИЙ, ФИБРИНОЛИТИЧЕСКИЙ И АНТИКОАГУЛЯНТНЫЙ ЭФФЕКТЫ У КРЫС С ГИПЕРГЛИКЕМИЕЙ

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Ранее показано, что применение регуляторных пептидов семейства глипролинов способствует нормализации системы гемостаза и уровня глюкозы крови при экспериментальной стойкой гипергликемии у крыс, подобной сахарному диабету 2 типа у человека. Также известно, что антикоагулянт гепарин подавляет свертывание крови и проявляет гипогликемическое действие в организме.

Цель исследования. Получить комплекс пептида Pro-Gly-Pro-Leu (PGPL) и нефракционированного гепарина, изучить его влияние на уровень глюкозы и антикоагулянтно-фибринолитические свойства и показать его способность восстанавливать нарушенные функции инсулярной и свертывающей систем крови при экспериментальной гипергликемии у крыс.

Материалы и методы. Использовались лабораторные крысы-самцы линии Wistar, интактные и с экспериментально вызванной гипергликемией. Было создано комплексное соединение PGPL и гепарина при соотношении компонентов 1: 1 (моль/моль), которое вводили один раз в сутки в течение 5 дней интраназально в дозе 1 мг/кг гипергликемическим крысам. Подобным образом вводили составные части комплекса в эквивалентных количествах. Определяли антикоагулянтную активность по тесту активированного частичного тромбопластинового времени, параметры фибринолиза по тестам суммарной, ферментативной и неферментативной фибринолитической активности, а также активности тканевого активатора плазминогена. Кроме того, проводилось измерение уровня глюкозы крови с использованием специальных тест-полосок.

Результаты. Применение комплекса PGPL-гепарин у животных с гипергликемией приводило к нормализации уровня глюкозы крови, повышению антикоагулянтного и фибринолитического фона плазмы крови. Эти эффекты сохранялись в течение 6 дней после прекращения введения крысам пептидно-гепаринового комплекса.

Заключение. Комплекс PGPL с гепарином проявляет комбинированное гипогликемическое, антикоагулянтное и фибринолитическое ферментативной и неферментативной природы действие при развитии экспериментальной гипергликемии. Исследуемый пептидно-гепариновый комплекс в перспективе может применяться для профилактики и лечения сахарного диабета 2 типа, осложняющегося повышенной свертываемостью крови.

Ключевые слова: антикоагулянтная активность; уровень глюкозы крови; комплексное соединение; фибринолиз; глипролиновые пептиды; гипергликемия; сахарный диабет 2 типа

INTRODUCTION

It is known that the incidence of type 2 diabetes mellitus (insulin-independent, T2DM) is constantly growing. Diabetes mellitus is a complex of physiological dysfunctions characterized by a persistent increase in blood glucose (hyperglycemia) due to insulin resistance, insufficient secretion of insulin and excessive secretion of glucagon into blood [1, 2]. Hyperglycemia and the development of diabetes mellitus are usually accompanied by anticoagulant system (ACS) dysfunction and hypercoagulation, which is manifested by increased platelet aggregation and a decreased anticoagulant and fibrinolytic blood activity [3, 4].

There is evidence that amino acids such as arginine, leucine play an important role in the prevention of diabetes mellitus. Leucine stimulates the production of insulin by pancreas, which leads to normal blood glucose levels in patients with diabetes mellitus [5, 6].

Short regulatory proline- and glycine-containing peptides (glyprolines) are constantly produced in the body during the synthesis and degradation of collagen [7]. Some glyprolines (Arg-Pro-Gly-Pro, Gly-Pro-Arg, Leu-Pro-Gly-Pro, Pro-Gly-Pro-Leu) exhibit a protective antidiabetogenic effect, meanwhile stabilizing hemostasis in metabolic disorders.

It has been shown that repeated intranasal admin-

istration of these peptides to rats with persistent hyperglycemia (similar to the initial stage of human T2DM) was leading to positive changes in the insular and coagulation systems of the body. At the same time, a decrease in the glucose level, an increase in the anticoagulant and fibrinolytic potential of plasma, as well as inhibition of platelet aggregation in the animal blood plasma, have been noted [3, 4, 8, 9].

One of the natural anticoagulants of the hemostatic system – heparin, is a component of the internal environment of the body, which is synthesized by mast cells of the liver, basophilic leukocytes and other cells. It belongs to fast-acting anticoagulants, inhibits a thrombin-coagulating activity, and also exhibits a hypoglycemic effect in the body. Heparin is able to form complexes with amino acids and peptides due to the presence (in its molecule) of structural zones of binding to these ligands [10, 11]. It has been experimentally shown that the administration of heparin complexes with glyprolines to the animals led to an increase in the anticoagulant, fibrinolytic and antithrombotic activity of the blood [12, 13].

It has been established that intranasal administration of a complex of heparin with the peptide Arg-Pro-Gly-Pro to the animals with hyperglycemia, increases the anticoagulant properties of blood plasma, fibrinolytic activity of an enzymatic and non-enzymatic nature, reduces platelet aggregation and protects rats from developing diabetes mellitus [13].

In this study, the hypoglycemic, anticoagulant and fibrinolytic effects of the complex compound Pro-Gly-Pro-Leu (PGPL) with unfractionated heparin were studied. That complex had been created and repeatedly administered by intranasal way to the animals with persistent hyperglycemia similar to T2DM in humans.

THE AIM of the study is to obtain a complex of the Pro-Gly-Pro-Leu (PGPL) peptide and unfractionated heparin, to study its effect on the level of glucose and on the anticoagulant and fibrinolytic properties and show its ability to restore the impaired functions of the insular and coagulating blood systems in experimental hyperglycemia in rats.

MATERIALS AND METHODS

Obtaining the PGPL-heparin complex

In this study, unfractionated heparin (Serva, Germany) was used. The PGPL peptide was synthesized at the Institute of Molecular Genetics, Russian Academy of Sciences (Moscow, Russia). A complex compound of this peptide with heparin at the component ratio of 1: 1 (mol / mol) was created according to the previously described protocol with some modifications [12].

Heparin and the PGPL peptide were dissolved in distilled water, mixed up and incubated for 1 h at 37° C, pH 7.2. The reaction product was precipitated with 1% acetic acid, pH 5.0–5.2, and centrifuged for 30 min. at 3000 × g. The precipitate was dissolved in 0.85% NaCl (physiological saline), pH 7.2–7.4.

Bond formation between heparin and peptide was assessed by cross-sectional electrophoresis at 20° C in 0.053 M phosphate buffer, pH 7.0, with a potential gradient of 7.3 w/cm for 2 hours. Then, the electrophoregrams were stained with a 0.033% solution of Azur II, which reveals the acid groups of heparin [12].

Animals, study design and drug administration

50 male Wistar rats (aged 10 months, weighing 320–350 g) were obtained from the Scientific Center for Biomedical Technologies, Stolbovaya branch (Moscow, Russia). The animals were kept in plastic cages under standard vivarium conditions at the ambient temperature of 21–24°C, a 12-hour day/night cycle and a relative humidity of 60 ± 10% with free access to food and water during the experiment.

All the experimental procedures were carried out in accordance with the ethical principles for the use of laboratory animals and were approved by the local ethics committee (Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, Russia).

After a 10 days' adaptation, all the animals were divided into 5 groups, each of 10 rats (Fig. 1).

Group 1 – rats with hyperglycemia, treated with 0.85% saline.

Group 2 – rats with hyperglycemia treated with the PGPL-heparin complex (1 mg/kg body weight).

Group 3 – rats with hyperglycemia treated with PGPL (20 µg/kg body weight).

Group 4 – rats with hyperglycemia treated with heparin (0.98 mg/kg body weight).

Group 5 consisted of healthy animals.

The preparations dissolved in a 0.85% sodium chloride solution (the PGPL-heparin complex compound, PGPL peptide and heparin), were prepared daily before the administration to the animals. The dose of the PGPL-heparin complex was selected on the basis of previous studies [13]. The doses of the components of the complex, i.e. the PGPL peptide and heparin, were equivalent to their content in the complex compound.

Experimental persistent hyperglycemia (HG) in the rats of groups 1–4 was induced by a daily single intragastric administration of a 40% glucose solution at the dose of 2.5 ml/kg body weight for 7 days, after which the rats of these groups continued to be treated with a glucose solution throughout the experiment. After the development of HG, the rats of groups 2–4 were intranasally injected with the studied drugs in the volume of 25 µl once a day for 5 days. At the same time, the rats of the 1st and 5th groups were treated with 0.85% NaCl as a placebo intranasally.

On the 22nd day of the experiment, 1 hour after the last injection of the drugs, the blood was taken from each animal for biochemical analyzes. At the end of the experiment (Day 28), repeated blood sampling was carried out 1 hour after the last glucose administration against the background of the cancellation of the drug administration.

Daily administration of a 40% glucose solution per os (Groups 1–4)									
Day 1									
Adaptation to vivarium conditions					Intranasal administration: Group 1 – 0,85% saline Group 2 – Complex Group 3 – PGPL Group 4 – Heparin Group 5 – 0,85% saline				
							1 st blood drawing		2 nd blood drawing

Figure 1 – Scheme of the experiment

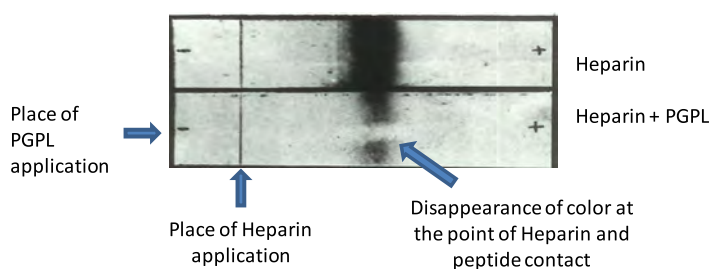


Figure 2 – Electrophoregrams of migration of heparin detached and heparin interacting with the peptide, in the electric field

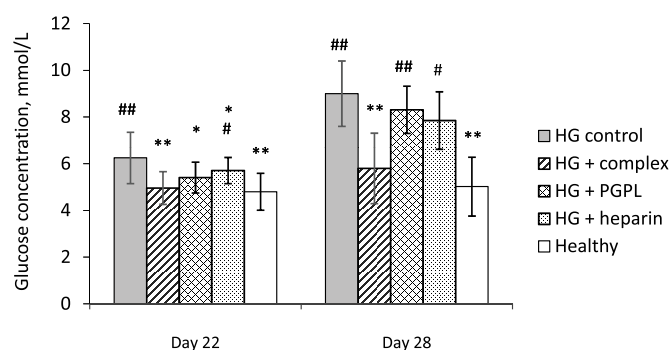


Figure 3 – Blood glucose level (mmol/l) on the 22nd day (1 hour after the 5th administration of drugs) and on the 28th day of the experiment (6 days after the cancellation of drug administration)

Note: * $P < 0.05$ and ** $P < 0.01$ – compared with the HG control (Group 1), # $P < 0.05$ and ## $P < 0.01$ – compared with healthy animals (Group 5).

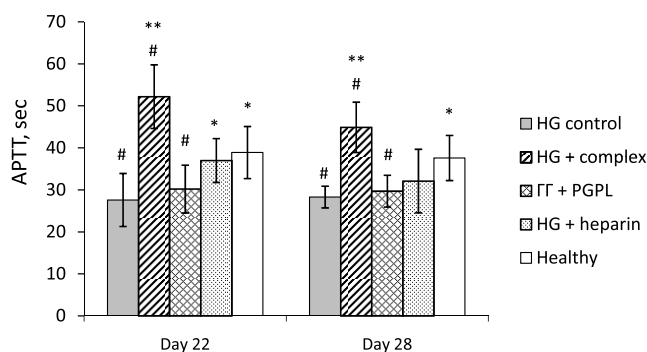


Figure 4 – Anticoagulant activity of blood plasma (according to the APTT test) on the 22nd day (1 hour after the 5th administration of drugs) and on the 28th day of the experiment (6 days after the cancellation of the drug administration)

Note: * $P < 0.05$ and ** $P < 0.01$ – compared with the HG control (Group 1), # $P < 0.05$ and ## $P < 0.01$ – compared with healthy animals (Group 5).

Conducting biochemical analyzes

For the analysis, the blood was taken from the jugular vein (*vena jugularis*), using 3.8% sodium citrate as a preservative in the ratio of blood : preservative as 9:1. In the whole blood, glucose concentration was determined by an Accutrend GC analyzer (Roche, Germany) using special test strips. Then, the blood was centrifuged at $2000 \times g$ for 15 min at room temperature to obtain platelet-poor plasma, in which an anticoagulant activity was assessed by the activated partial thromboplastin time (aPTT) test on an ASK 2-01 blood coagulation analyzer (Russia); fibrinolysis indicators according to tests of total (TFA), non-enzymatic (NFA) fibrinolytic activity on unstabilized fibrin, enzymatic fibrinolytic activity (EFA) and tissue plasminogen activator activity (PAA) in the euglobulin plasma fraction on stabilized fibrin [14].

All the obtained data were statistically processed and expressed as mean \pm SD (standard deviation). The normality of the distribution was revealed by the Shapiro-Wilk test, the group differences were analyzed by the method of one-way Analysis of Variants (ANOVA) using the Newman-Keuls-test for multiple comparisons. The values of $P < 0.05$ were considered statistically significant.

RESULTS

Preparation of the PGPL-Heparin Complex

The formation of a complex compound of the PGPL peptide with heparin in a pure system has been established. This compound was obtained with an optimal molar ratio of the components: 1:1. The use of cross-electrophoresis showed the interaction and the occurrence of a chemical bond between the amino groups of the PGPL peptide and the acid groups of heparin.

As Fig. 2 shows, when the solutions of heparin and PGPL were perpendicular to the carrier with their further migration in the electric field, the color of the acidic carboxyl and sulfate groups of heparin (Azur II dye) disappeared at the meeting point of the substances.

Next, the hypoglycemic effect and the effect on the hemostasis system of the PGPL-heparin complex compound and its components – the PGPL peptide and heparin in equivalent doses under conditions of persistent GH in rats – were studied.

The effect of the PGPL-heparin complex on blood glucose

On the 18th day of the experiment, after the induction of hyperglycemia, the blood glucose level in rats with GH (group 1) was significantly higher than the one in the healthy control (group 5) (6.25 ± 0.35 mmol/L versus 4.8 ± 0.25 mmol/L, respectively; $P < 0.01$), which amounted to 130%. The administration of the PGPL-hep-

arin complex compound to the rats decreased this indicator by 21% (4.96 ± 0.23 mmol/L; $P < 0.01$). The administration of the complex components (PGPL or heparin) had a weaker hypoglycemic effect, which led to a lower decrease in blood glucose levels by 13 and 9%, respectively (to 5.41 ± 0.21 mmol/L with PGPL and 5.71 ± 0.56 mmol/L with heparin; $P < 0.05$).

On the 28th day of the experiment (6 days after the treatment had been canceled), the blood glucose level in the rats of group 1 continued to increase and amounted to 179% relative to the healthy animals (group 5) (9.0 ± 0.42 mmol/L versus 5.02 ± 0.32 mmol/L; $P < 0.01$).

Meanwhile, the blood glucose level in group 2 rats treated with the complex, was 5.8 ± 0.72 mmol/L ($P < 0.01$), while the administration of both PGPL and heparin did not change this indicator ($P > 0.05$) (Fig. 3)

The effect of the PGPL-heparin complex compound on the anticoagulant activity of blood plasma by the APTT test

As Fig. 4 shows, on the 18th day of the experiment, in rats with HG (group 1) the anticoagulant activity of blood plasma was 27.6 ± 6.32 sec., i.e. it had been reduced by 29% compared with healthy normal rats ($P < 0.01$). This conclusion confirms that in HG animals, the procoagulant activity of blood increases 12 days after the induction of HG.

In HG rats, after therapy by the complex and heparin, the anticoagulant activity significantly increased by 89% and 34%, respectively (52.2 ± 7.58 sec. – group 2; $P < 0.01$ and 37.0 ± 5.22 sec. – group 4; $P < 0.05$), while the PGPL administration did not change the anticoagulant activity of blood plasma in the rats ($P > 0.05$).

On the 28th day of the experiment, i.e. 6 days after the cancellation of the drug administration, the anticoagulant blood activity significantly differed from the control group (28.3 ± 3.52 sec.) only in Group 2 after the administration of the PGPL-heparin complex (44.9 ± 5.01 s; $P < 0.01$) by 58% (Fig. 4). The introduction of the complex components (PGPL and heparin) did not lead to a significant change in this parameter ($P > 0.05$).

The effect of the PGPL-heparin complex compound on fibrinolytic activity (TFA, NFA, EFA and PAA) of blood plasma

The carried out experiment showed that in rats with GH (Group 1), the fibrinolytic activity of blood plasma was inhibited in comparison with healthy animals of Group 5 (Table 1). Thus, the indicators of TFA, NFA and EFA in HG animals were reduced by 19, 28 and 32%, respectively, compared with those in healthy rats ($P < 0.05$). In the rats of Group 1, PAA was also 36% lower than in the rats of Group 5 ($P < 0.01$).

Table 1 – Fibrinolytic activity (mm²) of blood plasma of animals on the 22nd day (1 hour after the 5th administration of drugs) and on the 28th day of the experiment (6 days after the cancellation of the drug administration)

Groups of animals	TFA	NFA	EFA	PAA
1 hour after the 5th administration of drugs (22 nd day)				
HG control (Group 1)	27.5 ± 5.05 ^{##}	16.7 ± 3.09 [#]	52.7 ± 11.3 [#]	17.8 ± 6.51 ^{##}
HG + complex (Group 2)	52.0 ± 10.1 ^{*,##}	35.8 ± 6.29 ^{*,#}	89.3 ± 8.54 ^{*,##}	49.4 ± 10.1 ^{*,##}
HG + PGPL (Group 3)	33.4 ± 6.24 [*]	21.8 ± 3.99	71.5 ± 5.25 [*]	24.1 ± 4.43 [*]
HG + heparin (Group 4)	31.8 ± 4.78	19.4 ± 4.6	68.7 ± 7.48 [*]	27.3 ± 6.15 ^{**}
Healthy (Group 5)	34.0 ± 5.98 ^{**}	23.1 ± 2.56 [*]	77.5 ± 12.4 [*]	28.0 ± 7.2 ^{**}
6 days after the cancellation of drug the administration (28th day)				
HG control (Group 1)	28.7 ± 2.83 ^{##}	17.3 ± 3.4 ^{##}	37.1 ± 8.36 ^{##}	9.4 ± 3.61 [#]
HG + complex (Group 2)	38.7 ± 5.22 ^{**}	25.8 ± 2.86 ^{**}	60.6 ± 4.9 ^{**}	22.6 ± 4.38 ^{**}
HG + PGPL (Group 3)	32.2 ± 4.44	20.7 ± 3.74	45.2 ± 5.31 ^{*,#}	15.7 ± 2.41 [*]
HG + heparin (Group 4)	30.7 ± 6.27 [#]	22.8 ± 4.18	42.1 ± 6.06 ^{##}	12.5 ± 4.6
Healthy (Group 5)	39.6 ± 4.33 ^{**}	25.0 ± 3.97 ^{**}	65.8 ± 5.05 ^{**}	19.0 ± 5.33 [*]

Note: * $P < 0.05$ and ** $P < 0.01$ – compared with group 1 (HG control), # $P < 0.05$ and ## $P < 0.01$ – compared with group 5 (healthy rats). The data are presented as $M \pm SD$. TFA – Total fibrinolytic activity, NFA – Non-enzymatic fibrinolytic activity, EFA – Enzymic fibrinolytic activity, PAA – t-Plasminogen activator activity.

After the intranasal administration of the PG-PL-heparin complex, PGPL peptide and heparin, the fibrinolytic activity of blood plasma in the rats with resistant HG led to an increase of fibrinolysis in all types (TFA, NFA, EFA, PAA), but to different extents. So, on the 22nd day of the experiment, in rats of group 2 treated with the complex, these indicators increased by 89, 115, 69 and 177% ($P < 0.01$), respectively, compared with the control group (Group 1). Under these circumstances, fibrinolysis parameters exceeded those in healthy animals (Group 5). At the same time, the use of peptide led to a significant increase in TFA by 21% ($P < 0.05$), EFA – by 36% ($P < 0.05$) and PAA – by 35% ($P < 0.05$) in the blood plasma of rats of group 3, but it did not change NFA in comparison with the HG control ($P > 0.05$). The administration of heparin caused a significant increase in EFA alone – by 30% ($P < 0.05$) and PAA – by 53% ($P < 0.01$) in the rats of group 4 compared with the control group.

On the 28th day of the experiment, in rats with GH (group 1), a reduced background of fibrinolysis remained: TFA, NFA, EFA ($P < 0.01$ in all cases) and PAA ($P < 0.05$) were significantly lower (by 27, 30, 44 and 51%, respectively) than in healthy animals (Group 5). At the same time, in group 2, even after the cancellation of the complex administration, a statistically significant increase in fibrinolytic activity was noted: TFA – by 35%, NFA – by 49%, EFA – by 63% and PAA – by 140% compared with the HG control group ($P < 0.01$). These indicators corresponded to the values of healthy control. In addition, in group 3 (PGPL), there was a significant increase in only EFA (by 22%, $P < 0.05$) and PAA (by 67%, $P < 0.05$). Under these circumstances, in group 4, after the cancellation of the heparin administration, there were no significant differences in fibrinolytic activity compared with the HG control ($P > 0.05$).

DISCUSSION

Analyzing the obtained results, it should be noted that the progression of diabetes mellitus is accompanied by a suppression of inhibition of insular [15] and anticoagulation systems. The depression of ACS is characterized by increased platelet aggregation, a decreased anticoagulant and fibrinolytic activity of plasma hemostasis, which leads to an increase in blood thrombogenic potential, and also often causes blood clots [8].

According to the literature data, in HG rats the suppression of the ACS function is accompanied by an increase in blood coagulability, a decrease in fibrinolysis, and a significant increase in blood glucose levels [3, 4, 13]. A similar effect was observed in our model of experimental hyperglycemia.

In this study, the effect of the PGPL-heparin complex compound and its components – heparin and PGPL peptide – on the hemostatic and insular systems in HG rats was evaluated. It was shown that in the situation where persistent hyperglycemia developed, the PGPL-heparin complex had a protective effect on the functional state of both the insular and hemostatic systems of the body, which had been identified for the first time.

Both PGPL peptide and heparin in the doses equivalent to their content in the complex, stimulated the anticoagulant and fibrinolytic activity of blood and lowered glucose levels. It was established that the anticoagulant effects of the complex exceeded the action of its constituent parts: as a result of the activation of NFA and a tissue plasminogen activator, TFA and anticoagulant activities of the blood got increased in the blood plasma. In addition, the effect of the PGPL-heparin complex was prolonged, since it persisted 6 days after the cancellation of its administration.

Previously, the effect of the peptides PG, PGP and RPPG, heparin and their complex compounds were

evaluated in healthy and hyperglycemic rats. In the rats treated with heparin intranasally, only an anticoagulant activity increased, and when the peptides were administered, both anticoagulant and fibrinolytic properties of the blood increased [12, 13, 16]. A repeated intranasal administration of the PGPL peptide at the higher dose (1 mg/kg) than in the present study, to rats with persistent GH, protected the body from the development of hypercoagulation. At the same time, there was an increase in total and non-enzymatic fibrinolysis of the animals' blood. Positive changes in the insulin and anticoagulation systems were observed within 5 days after the cancellation of the peptide administration [9].

Considering the mechanism of the effect of the complex on the normalization of blood glucose levels, it can be assumed that the PGPL-heparin had this effect and prevented the development of T2DM due to the presence of the amino acid leucine in its structure, which stimulates the production of insulin, providing a normoglycemic effect [6].

The mechanism of the anticoagulant effect of heparin is due to the blockade of the activity of thrombin and other coagulant proteins [17]. At the same time, heparin increased the activity of the tissue plasminogen activator, which led to the increase of enzymatic fibrinolysis [10].

Some peptides are effective thrombin inhibitors that have anticoagulant properties, can activate enzymatic fibrinolysis and prevent forming blood clots. It was established that proline and glycine-containing peptides PG and PGP can induce dissolution of the unstabilized fibrin [16]. The presence of glyproline receptors on the endothelium has not been established yet, but specific PGPL binding sites on the cytoplasmic membrane of the rats'

brain basal nuclei have been reported [18]. Perhaps this peptide, administered by the intranasal route, passes through the blood-brain barrier, penetrating the structures of the brain and exerting its action through specific receptors. According to the literature data [19, 20], as well as according to the results of this study, individual peptides are able of exerting a hypoglycemic effect and inhibit the progression of T2DM.

CONCLUSION

The PGPL-heparin complex studied by the carried out experiment, enhanced the hypoglycemic and anticoagulant effect of its constituent components, heparin and peptide, due to its structural features. It prevented the formation of fibrin due to the presence of its fibrinolytic activity, determined by its anticoagulant effect on the thrombin activity. The appearance of both PGPL and the complex in the bloodstream, stimulates the release of the tissue plasminogen activator from the vascular endothelium, which leads to an increase in the enzymatic fibrinolytic properties of the blood, and the effect of the complex is more effective. It has also been shown that the complex significantly improves functioning of the insular system in hyperglycemic rats, and its component heparin modulates the function of the anticoagulant system, participating in the prevention of the hypercoagulation process that accompanies the development of T2DM.

In the future, the obtained results on the model of the rats with persistent hyperglycemia, may be applicable in clinical practice for patients with type 2 diabetes who are predisposed to the development of thrombotic complications.

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AUTHOR'S DEPOSIT

All the authors have equally contributed to the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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