УДК 577.1+616.379-008.64+615.27

LVV-hemorphin-3 affects secretion and plasma concentrations of insulin and glucagon in streptozotocin-induced diabetic rats

F.P. Sarukhanyan, O.V. Hunanyan, N.H. Barkhudaryan

H. Buniatian Institute of Biochemistry NAS RA
5/1 P. Sevag str., 0014, Yerevan, Armenia

Keywords: hemorphin, diabetes, insulin, glucagon

The prevalence of diabetes mellitus is increasing dramatically throughout the world. Numerous efforts are continually being made to study diabetes and diabetes-related complications, to test new drugs and to develop new strategies in preventing and treating diabetes.

In our previous studies we have shown that intraperitoneal (ip) injection of LVV-hemorphin-3 (LVV-H3) into streptozotocin (STZ)-induced diabetic rats causes a statistically significant decrease in plasma glucose concentration in dose-dependent manner [4]. LVV-H3, also known as myelopeptide-2, is a member of hemorphins family, the endogenous non-classical opioid peptides derived from hemoglobin (for review see Ref.20). Parallel to plasma glucose-lowering effect, LVV-H3 induces an increase in plasma β-endorphin-like immunoreactivity [4]. It is necessary to emphasize that metformin, which was successfully used in clinical treatment of type 2 diabetes, also decreased the plasma glucose of STZ-induced diabetic rats with a parallel increase of plasma β-endorphin-like immunoreactivity [5].

Furthermore, we have established that Ca^{2+}/CaM-dependent serine/threonine protein phosphatase calcineurin signaling pathway and μ-opioid receptors (MOR) are involved in the molecular mechanisms of anti-diabetic effect of LVV-H3 [4]. We have also shown that LVV-H3 is able to recover increased calcineurin activity and neuro-immune-endocrine changes in STZ-induced diabetic rats [21]. Calcineurin controls gene expression of several cytokines, including IL-2 and tumor necrosis factor α (TNFα), and other regulatory proteins via dephosphorylation and nuclear translocation of NFATc (nuclear factor of activated T cell) family members [11]. The involvement of cytokines, such as IL-2 and TNFα, in insulin signaling was also reported [7].

It should be emphasized that calcineurin/NFAT signaling pathway was shown to be involved in regulation of insulin and insulin-like growth factor-1(IGF-1) genes expression [3, 16], and modulation of insulin receptor (IR)

function. In addition, insulin receptor substrate-1 (IRS-1) is considered as direct substrate for calcineurin in vivo [11]. Very recently it has been reported that specific glucose-induced increase in IRS-2 expression in primary islet β-cells is also mediated by activation of calcineurin/NFAT pathway [8]. Thus, calcineurin interacts with all components of insulin system (insulin, IR, IGF-1, IRS-1, IRS-2) both at transcriptional level and posttranslational modification.

Another key hormone in the regulation of glucose homeostasis – glucagon acts as a counter-regulatory hormone to insulin by promoting hepatic glucose output. In diabetes, while insulin secretion or action is insufficient, the production and secretion of glucagon are excessive, contributing to the development of diabetic hyperglycemia [24]. On the basis of the concept that glucose metabolism and homoeostasis are dually controlled by the pancreatic insulin and glucagon, rather than insulin alone, the pharmaceutical industry has attempted to develop potent and selective glucagon receptor antagonists. The glucagon receptor antagonists THG [14] and des-His1-[Glu9] glucagon amide [23] were reported to inhibit glucagon-induced hyperglycemia or lowered blood glucose in STZ-induced diabetic rats. A unique class of new anti-diabetic drugs, DPP-IV (dipeptidyl peptidase IV) inhibitors, was shown to exhibit both insulin-promoting and glucagon suppressing effects [1, 17]. It should be noted that hemorphins are inhibitors of DPP-IV as well [6].

Though the plasma glucose-lowering effect of LVV-H3 was reported [4], however, till now it is not clear if hemorphins may affect insulin and glucagon secretion and plasma concentrations of these hormones in diabetes. Therefore, in the frame of this study we have determined the changes in secretion of insulin and glucagon in pancreatic islets and revealed the changes in plasma concentrations of these hormones in STZ-induced diabetic rats, received ip administration of hemorphin.

Materials and Methods

In the experiments male rats (Wistar line) were used, weighing 180-200g. Rats provided by UNESCO Chair-Life Science International Postgraduate Educational Center (LSIPEC, Yerevan, Armenia), were caged in groups of 5 with food and water given ad libitum. Animals were kept at 22°C on a 12 h light-dark cycle. Diabetes was induced by single ip injection of 60 mg/kg body weight streptozotocin (STZ) (Sigma-Aldrich Inc., USA) made in fresh 0.1 M citrate buffer, pH 4.5. The rats in control group (n=5) received ip injection of an equivalent volume (0.5 ml) of 0.9% w/v saline. Glucose concentration in the blood, obtained from the tail vein of fasted rats under the light halothane anaesthesia, was measured using the GlucoPlus glucometer (GlucoPlus Inc., Canada). Animals were considered diabetic if they had blood glucose concentrations >20 mmol/l in additional to polyuria and other diabetic features. Two weeks after treatment with STZ, rats were randomly divided into 3 groups:

1/ diabetic untreated group received ip saline injection (n=5); 2/ LVV-H3-treated diabetic group, which received a single ip injection of 1mg/kg synthetic LVV-H3 (ApexBio Inc., USA) dissolved in saline (n=7); 3/ LVV-H3-treated diabetic group, which received daily a single ip injection of 1mg/kg LVV-H3 during 5 days (n=7).

Rats in the 2-nd group were decapitated under the deep halothane anaesthesia 1h after hemorphin injection, since in previous studies we have demonstrated that LVV-H3 at the doses 1 mg/kg exhibit maximal blood glucose-lowering effect 1h after administration [1]. Rats in the third group were decapitated 5 days after hemorphin treatment. The trunk blood was collected immediately into sodium citrate (3.2%)-coated vacutainer tubes. Blood samples were centrifugated at 1500 rpm for 10 min, plasma was separated into aliquots and stored at -70°C for further investigations by the ELISA.

Isolation of pancreatic islets. Pancreatic islets were isolated by the method of Lacy and Kostianovsky [15] with slight modifications. After decapitation of the rats, pancreases were removed rapidly by collagenase digestion. Collagenase (CLS IV, Biochrom GmbH, Germany) was dissolved (1,000 U ml^{-1}) in 1× HBSS (Hank's balanced salt solution, 8 ml for each rat). After an incision was made around the upper abdomen to expose the liver and intestines, 4 ml of collagenase solution was slowly injected into the common bile duct to distend the pancreas. The pancreas was removed and placed in a 50 ml tube containing 4 ml of collagenase solution and incubated in a water bath at 37.5 °C for 15 min. After incubation, the tube was shaken by hand to disrupt the pancreas until the suspension turned homogeneous, and the digestion was terminated by putting the tube on ice and adding 25 ml of 1×HBSS, containing 1 mM $CaCl_2$. The supernatant was discarded after centrifugation at 290*g* for 30 sec. at 4 °C. This step was repeated once more, after which the resulting pellet was resuspended with 15 ml of Krebs-Ringer bicarbonate buffer (pH 7,4) containing 10 mM HEPES, 0,1% BSA and 1 mM glucose. After 30 min preincubation in this medium at 37°C, glucose-induced insulin and glucagon static secretion procedures were followed.

Insulin and glucagon secretion from pancreatic islets. In vitro studies were performed to compare the hormone-secretory response of pancreatic islets isolated from non-diabetic, diabetic and hemorphin-treated diabetic rats. The isolated islets were counted under light microscope and 10 islets were placed in 1 ml Krebs-Ringer bicarbonate buffer (pH 7,4) containing 10 mM HEPES, 0,1% BSA and low (7 mM) or high (20 mM) glucose solutions, respectively. 1 h after incubation at 37°C, the islets were sedimented and the conditioned medium was collected and frozen at -70°C for insulin and glucagon measurements by ELISA.

Determinations of insulin and glucagon. The released insulin and glucagon amounts in the pancreatic islets, as well as, the concentrations of insulin and glucagon in the plasma were determined by ELISA method, using

High sensitive rat Insulin ELISA kit (Biorbyt Ltd., UK) and Glucagon EIA kit (Biorbyt Ltd., UK). Mesurments were made according to the manufacturer's instructions. The optical density was measured at the wavelength of 450 nm using LABLine-022 microplate reader (LABLINE Diagnostics, Austria).

Statistical analysis. Data were analyzed statistically by one-way ANOVA using GraphPad Prism 4 software. Statistical significance – $p<0.05$. All data were expressed as means ± SEM.

Results and Discussion

It is well known that under normal conditions, insulin and glucagon operate in concert to maintain the glucose level within a narrow physiological range. In diabetes, however, while insulin secretion or action is insufficient, the production and secretion of glucagon are excessive, contributing to the development of diabetic hyperglycemia [2, 13, 24]. Similarly, the results obtained in our studies have demonstrated that STZ-induced diabetes in rats results in a significant decrease of plasma insulin (1.76 fold) and significant increase of plasma glucagon (1.9 fold) compared with plasma hormone concentrations of saline-treated healthy control rats (Fig. 1).

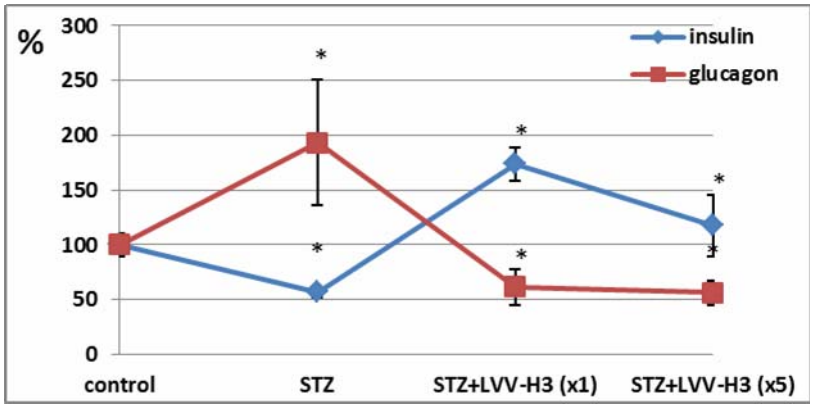

Fig. 1. Plasma insulin and glucagon levels in control, diabetic (STZ) and hemorphin treated diabetic rats. Insulin and glucagon levels were measured 1h after a single hemorphin ip injection and after ip injections during 5 consecutive days. Values represent means ± SEM. * - means differ statistically ($p<0.05$) between control and diabetic rats and treated and non-treated diabetic rats.

Plasma insulin was found to be increased by 3 times in diabetic rats 1h after a single LVV-hemorphin-3 (1 mg/kg) ip injection, whereas plasma glucagon was decreased by 3.1 times in these rats, compared with non-treated diabetic rats (Fig. 1). So far as we had demonstrated that LVV-H3 at the doses 1 mg/kg exhibit maximal blood glucose-lowering effect 1h after injection [4], the plasma hormones concentrations were measured also 1h after hemorphin

administration. Similarly, in this study 1h after hemorphin injection blood glucose in diabetic rats was significantly decreased from 29.3±1.5 mmol/l to 19.4±0.9 mmol/l. We observed the same picture also in case of LVV-H3 (1 mg/kg) ip injections during 5 consecutive days: plasma insulin was significantly increased by 2.7 times and plasma glucagon was significantly decreased by 3.4 times (Fig.1). The blood glucose was also decreased (20.03±1.3 mmol/l) compared with non-treated diabetic rats (29.28±1.09 mmol/l).

The glucose-induced insulin and glucagon static secretion procedures were performed to investigate the hormones secretion in pancreatic islets of diabetic rats under hemorphin treatment. Exposure of pancreatic islets isolated from control and diabetic rats to low (7 mM) and high (20 mM) glucose conditions induced concentration-dependent changes in insulin secretion compared to basal secretion measured in the presence of 1 mM glucose (Fig. 2). These results indicate that pancreatic islets isolated from both groups of animals were metabolically active and effectively responded to stimulation by glucose. Although basal insulin output, measured at 1 mM glucose, did not differ significantly, insulin secretion induced by 7 mM glucose diminished by 2.3 times in islets of diabetic rats compared to control islets (Fig. 2). Similarly, the insulin-secretory response to 20 mM glucose was significantly impaired in islets of diabetic animals, since these islets released by 4.7 times less insulin than those of the control rats (Fig. 2). It was expected, since in vitro studies demonstrated that glucose-induced insulin secretion is diminished especially at higher concentrations of the sugar [18-19]. Contrary to that, a significant increase in glucagon secretion was observed in islets of diabetic rats, induced by 7 mM and 20 mM glucose (1.25 fold and 4 fold, respectively), in comparison with control rats (Fig. 3b).

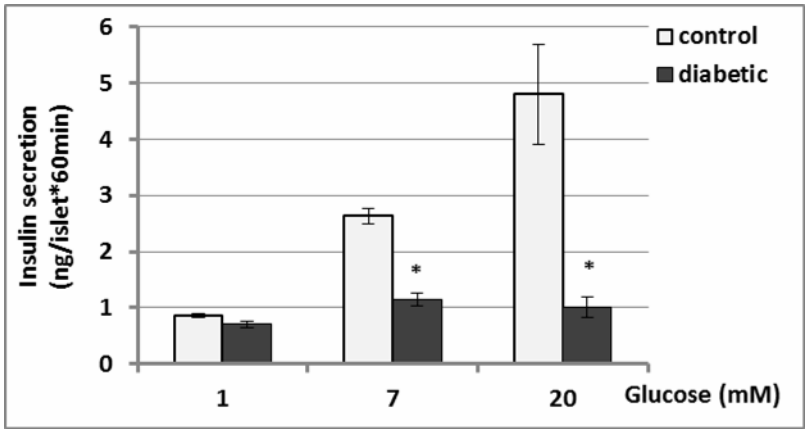

Fig. 2. Insulin secretion from pancreatic islets of control (white bars) and diabetic (black bars) rats induced 1h after incubation in 1 mM, 7 mM and 20 mM glucose conditions. Values represent means±SEM. * - means differ statistically ($p<0.05$) between control and diabetic rats.

It was demonstrated that after a single hemorphin injection to diabetic rats the islet insulin secretion induced by 7 mM glucose was significantly increased by 71.5% compared with non-treated diabetic rats (Fig. 3a), whereas elevated glucagon secretion in diabetic islets was completely abolished (Fig. 3b). Similarly, in these islets insulin secretion induced by 20 mM glucose was significantly increased by 45.6% (Fig. 3a) compared with non-treated diabetic rats, and glucagon secretion was significantly decreased by 25.5% (Fig. 3b). We found that long-term hemorphin treatment (5 consecutive days) caused moderate, but statistically significant changes in islet insulin and glucagon secretions. In these islets insulin secretion induced by 7 mM glucose was increased by 14% (Fig. 3a) and glucagon secretion was decreased by 16.2% (Fig. 3b) compared to non-treated diabetic rats. In 20 mM glucose condition insulin secretion was enhanced by 20.5% (Fig. 3a) and glucagon secretion was reduced by 42.7% (Fig. 3b) compared to non-treated diabetic animals.

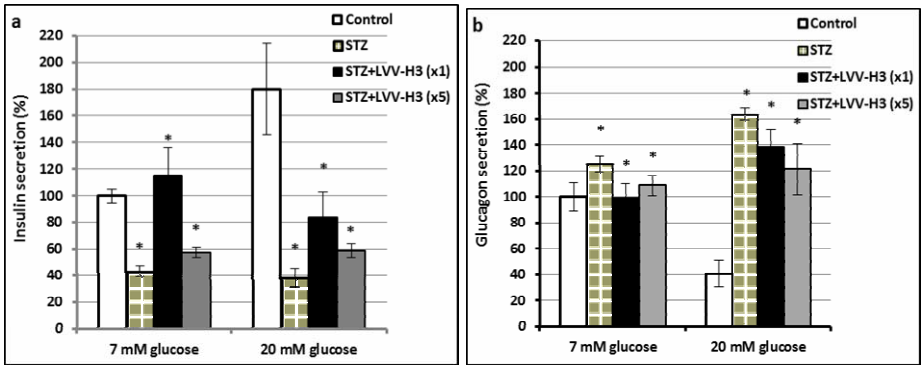

Fig. 3. **a.** Insulin secretion induced by glucose from pancreatic islets of control, diabetic and hemorphin-treated diabetic rats (single, ip injection – black bars and ip injections during 5 consecutive days – grey bars). **b.** Glucagon secretion induced by glucose from pancreatic islets of control, diabetic and hemorphin-treated diabetic rats (single ip injection – black bars and ip injections during 5 consecutive days – grey bars). Values represent means±SEM. * – means differ statistically ($p<0.05$) between control and diabetic rats and treated and non-treated diabetic rats.

Thus, as one can see, STZ-induced diabetes in rats significantly reduced plasma insulin concentration and its release from pancreatic islets due to β-cell destruction. Contrary to insulin, plasma glucagon concentration and its secretion from pancreatic islets is increased significantly in STZ-induced diabetic rats. These results correlate with other publications [13, 24].

We have found out that hemorphin regulates insulin and glucagon release in pancreatic islets as well as these hormones levels in plasma. The effect of LVV-H3 in all cases has been more pronounced 1h after injection, than after 5 consecutive days administration. Perhaps, this is a fast reaction of the organism to the hemorphin administration, and after 5 consecutive injections the effect of hemorphin reaches the plateau.

In our previous studies we have shown that LVV-H3 induces an increase in plasma β-endorphin-like immunoreactivity and recovers increased calcineurin activity in STZ-induced diabetic rats [4]. It should be noted that β-endorphin regulates the release of insulin and glucagon from pancreatic islets [9]. It has also been reported that β-endorphin increases insulin secretion by activation of opioid receptors located on β-cells of the pancreas [10]. Moreover, exogenous β-endorphin induced significant increase in circulating insulin in healthy individuals as well as in diabetic patients [10]. Calcineurin was shown to be a regulator of β-cells proliferation, mass and survival in part through the regulation of IRS-2 [8,12]. The functional link between glucagon and calcineurin in induction of gluconeogenic program was reported [25]. Taking into account all above mentioned, it seems very likely that LVV-H3 regulates insulin and glucagon secretion in pancreatic islets and modulates these hormones concentrations in plasma of diabetic rats by release of β-endorphin into circulation and by regulation of calcineurin activity.

The current study is the first to determine hemorphin-induced changes in insulin and glucagon secretion in isolated islets from diabetic animals. Interestingly, β-casomorphin-7, a member of the other endogenous non-classical opioid peptides family derived from β-casein, was reported to reduce blood glucose and glucagon levels, and increase insulin level in STZ-induced diabetic rats as well [26].

The data obtained in this study provide new evidence about antidiabetic nature of hemorphin and expand our knowledge concerning the role of hemorphins as members of endogenous protective system of the organism that recover the homeostatic disturbance in pathophysiology of diabetes.

Acknowledgment: This work was made possible by a research grant (Biochem-3568) from the Armenian National Science and Education Fund (ANSEF) based in New York, USA.

Поступила 07.09.15

LVV-հեմորֆին-3-ը ազդում է ինսուլինի և գլյուկագոնի սեկրեցիայի և պլազմայում դրանց կոնցենտրացիայի վրա ստրեպտոզոտոցինով խթանված դիաբետիկ առնետների մոտ

Ֆ.Պ. Սարուխանյան, Օ.Վ. Հունանյան, Ն.Հ. Բարխուդարյան

Ավելի վաղ մենք հայտնաբերել ենք LVV-հեմորֆին-3-ի (LVV-H3) գլյուկոզ նվազեցնող ազդեցությունը ստրեպտոզոտոցինով (STZ) խթանված դիաբետիկ առնետների պլազմայում, սակայն, պարզ չէ, թե արդյոք LVV-H3-ը կարող է ազդել պլազմայում ինսուլինի և գլյուկագոնի մակարդակների վրա: Այս աշխատանքում ELISA մեթոդով

ուսումնասիրվել են հեմորֆինի (1 մգ/կգ) ներորովայնային ներարկումներ ստացած STZ-ով խթանված դիաբետիկ առնետների ենթաստամոքսային գեղձի կղզյակներից ինսուլինի և գլյուկագոնի սեկրեցիայի և պլազմայում դրանց կոնցենտրացիաների փոփոխությունները: Արդյունքները ցույց են տվել, որ առողջ առնետների համեմատ ինսուլինի կոնցենտրացիան STZ-ով խթանված դիաբետիկ առնետների պլազմայում զգալիորեն նվազում է (1.76 անգամ), իսկ գլյուկագոնի կոնցենտրացիան՝ աճում (1.9 անգամ): Նմանապես խանգարվում է նաև դիաբետիկ առնետների ենթաստամոքսային գեղձի կղզյակներից այդ հորմոնների սեկրեցիան: LVV-H3-ի մեկանգամյա ներարկումից հետո դիաբետիկ առնետների պլազմայում ինսուլինի մակարդակը աճում է 3 անգամ, իսկ գլյուկագոնի մակարդակը՝ նվազում 3.1 անգամ: Հեմորֆինը կարգավորում է նաև այս հորմոնների սեկրեցիան ենթաստամոքսային գեղձի կղզյակներից:

Ուսումնասիրվել է նաև LVV-H3-ի ազդեցությունը նույն ցուցանիշների վրա 5-օրյա ներարկումների արդյունքում: Այս դեպքում ևս հեմորֆինը կարգավորում է դիաբետիկ առնետների պլազմայում ինսուլինի և գլյուկագոնի մակարդակները, ինչպես նաև՝ դրանց սեկրեցիան: Այսպիսով, մենք պարզել ենք, որ LVV-H3-ը, ազդելով ենթաստամոքսային գեղձի կղզյակներից ինսուլինի և գլյուկագոնի սեկրեցիայի վրա, կարգավորում է դրանց մակարդակները պլազմայում՝ այդպիսով դրսևորելով գլյուկոզ նվազեցնող ազդեցություն դիաբետիկ առնետների մոտ:

Աշխատանքում ստացված տվյալները LVV-H3-ի հակաշաքարախտային հատկության նոր ապացույցներ են ներկայացնում և ընդլայնում են մեր գիտելիքները հեմորֆինների դերի մասին, որպես օրգանիզմի էնդոգեն պաշտպանական համակարգի անդամների, որոնք վերականգնում են շաքարախտի պաթոֆիզիոլոգիայում խաթարված հոմեոստազը:

LVV-геморфин-3 влияет на секрецию и концентрацию инсулина и глюкагона в плазме у стрептозотоцин-индуцированных диабетических крыс

Ф.П. Саруханян, О.В. Унанян, Н.А. Бархударян

Ранее нами был выявлен глюкозопонижающий эффект LVV-гемофина-3 (LVV-H3) в плазме у стрептозотоцин (STZ)-индуцированных диабетических крыс, однако, не было ясно, может ли LVV-H3 повлиять на уровни инсулина и глюкагона в плазме. В данной работе ELISA методом

были изучены изменения секреции инсулина и глюкагона в панкреатических островках и их концентрации в плазме у STZ-индуцированных диабетических крыс, получивших внутрибрюшинные инъекции геморфина (1 мг/кг). Полученные результаты показали, что концентрация инсулина в плазме STZ-индуцированных диабетических крыс значительно снижается (в 1,76 раза), а концентрация глюкагона увеличивается (в 1,9 раза) по сравнению со здоровыми крысами. Подобным образом нарушается также секреция этих гормонов из панкреатических островков диабетических крыс. После однократной инъекции LVV-H3 уровень инсулина в плазме диабетических крыс увеличивается в 3 раза, а уровень глюкагона снижается в 3,1 раза. Геморфин также регулирует секрецию этих гормонов в панкреатических островках диабетических крыс. Было изучено воздействие LVV-H3 на те же показатели в результате однократных инъекций в течение 5 дней. В этом случае геморфин также регулирует уровни инсулина и глюкагона в плазме и модулирует их секрецию в панкреатических островках диабетических крыс. Таким образом, мы выяснили, что LVV-H3 проявляет глюкозопонижающий эффект, модулируя секрецию инсулина и глюкагона в панкреатических островках и тем самым регулируя уровни инсулина и глюкагона в плазме диабетических крыс.

Данные, полученные в этом исследовании, выявляют новые доказательства антидиабетического свойства LVV-H3 и расширяют наши знания о роли геморфинов как членов эндогенной защитной системы организма, который восстанавливает нарушенный гомеостаз при патофизиологии диабета.

References

1. *Ahren B., Landin-Olsson M., Jansson P.A. et al.* Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. J. Clin., Endocrinol., Metab., 2004, v. 89, p. 2078–84.
2. *Akbarzadeh A., Norouzian D., Mehrabi M.R. et al.* Induction of diabetes by streptozotocin in rats. Indian Journal of Clinical Biochemistry, 2007, 22 (2), p. 60-64.
3. *Alfieri C.M., Evans-Anderson H.J., Yutzey K.E.* Developmental regulation of the mouse IGF-I exon 1 promoter region by calcineurin activation of NFAT in skeletal muscle. Am. J. Physiol. Cell Physiol., 2007, v. 292, p. C1887-C1894.
4. *Barkhudaryan N., Sarukhanyan F., Kellermann J., Lottspeich F.* Calcineurin and μ-opioid receptors are involved in the molecular mechanisms of anti-diabetic effect of LVVYPW. Medical Science of Armenia, 2011, т. LI, 3, p. 33-42.
5. *Cheng J-T., Huang C.-C., Liu IM., Tzeng T.F., Chang C.J.* Novel mechanism for plasma glucose-lowering action of metformin in streptozotocin-induced diabetic rats. Diabetes, 2006, v. 55, p. 819-825.
6. *Cohen M., Fruitier-Arnaudin I., Piot J.M.* Hemorphins: substrates and/or inhibitors of dipeptidyl peptidase IV: Hemorphins N-terminus sequence influence on the interaction between hemorphins and DPPIV. Biochimie, 2004, v. 86, p. 31–37.
7. *Csehi S-B., Mathieu S., Seifert U.et al.* Tumor necrosis factor (TNF) interferes with insulin signaling trough the p55 TNF receptor death domain. Biochemical and Biophysical Research Communications, 2005, v. 329, p. 397-405.

8. *Demozay D., Tsunekawa S., Briaud I., Shah R., Rhodes C.J.* Specific Glucose-Induced Control of Insulin Receptor Substrate-2 Expression Is Mediated via Ca2+-Dependent Calcineurin/NFAT Signaling in Primary Pancreatic Islet β-Cells. Diabetes, 2011, v. 60, p. 2892–2902.
9. *Eigler N., Sacca L., Sherwin R.S.* Synergistic interactions of physiologic increments of glucagon, epinephrine, and cortisol in the dog. A model of stress-induced hyperglycemia. J. Clin. Invest., 1979, v. 63, p. 114–123.
10. *Giugliango D.D., Cozzolino D., Salvatore T., Ceriello A., Torella R.* Dual effect of beta-endorphin on insulin secretion in man. Horm. Metab. Res., 1987, v. 19, p. 502–503.
11. *Hallak H., Ramadan B., Rubin R.* Tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) by oxidant stress in cerebellar granule neurons: modulation by N-methyl-D-aspartate through calcineurin activity. Journal of Neurochemistry, 2001, v. 77, p. 63-70.
12. *Heit J.J., Apelqvist A.A., Gu X., Winslow M.M., Nelson J.R. Kim S.K.* Calcineurin/NFAT signalling regulates pancreatic β-cell growth and function. Nature Letters, 2006, v. 443, p. 345-349.
13. *Huang Y.C., Rupnik M. S., Karimian N. et al.* In Situ Electrophysiological Examination of Pancreatic α Cells in the Streptozotocin-Induced Diabetes Model, Revealing the Cellular Basis of Glucagon Hypersecretion. Diabetes, 2013, 62(2), p. 519–530.
14. *Johnson D.G., Goebel C.U., Hruby V.J., Bregman M.D., Trivedi D.* Hyperglycemia of diabetic rats decreased by a glucagon receptor antagonist. Science, 1982, v. 215, p. 1115–1116.
15. *Lacy P. E and Kostianovsky M.* Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes, 1967, v. 16, p. 335–339.
16. *Lawrence M.C., Bhat H.S., Watterson J.M., Easom R.A.* Regulation of insulin gene transcription by a Ca2+-responsive pathway involving calcineurin and nuclear factor of activated T cells. Mol. Endocrinol., 2001, v. 15, p. 1758-1767.
17. *Mari A., Sallas W.M., He Y.L. et al.* Vildagliptin, a dipeptidyl peptidase-IV inhibitor, improves model-assessed β-cell function in patients with type 2 diabetes. J. Clin. Endocrinol. Metab., 2005, v. 90, p. 4888–4894.
18. *Masiello P.* Animal models of type 2 diabetes with reduced pancreatic β-cell mass. Int. J. Biochem. Cell. Biol.**,** 2006, 38, p. 873-893.
19. *Novelli M., Fabregat M.E., Fernandez-Alvarez J., Gomis R., Masiello P.* Metabolic and functional studies on isolated islets in a new rat model of type 2 diabetes. Mol. Cell. Endocrinol., 2001, 175, p. 57-66.
20. *Nyberg F., Sanderson K., Glamsta E.L.* The hemorphins: a new class of opioid peptides derived from the blood protein haemoglobin. Biopolymers, 1997, v. 43, p. 147–156.
21. *Sarukhanyan F.P., Barkhudaryan N.H.* The regulatory influence of LVVYPW on immune-neuro-endocrine changes in streptozotocin-induced diabetes. Medical Science of Armenia, 2011, v. LI, 2, p. 63-71.
22. *Serfling E., Berberich-Siebelt F., Chuvpilo S. et al.* The role of NF-AT transcription factors in Tcell activation and differentiation. Biochim. Biophys. Acta, 2000, v. 1498, p. 1–18.
23. *Unson C.G., Gurzenda E.M., Merrifield R.B.* Biological activities of des-His1[Glu9]glucagon amide, a glucagon antagonist. Peptides, 1989, v. 10, p. 1171–1177.
24. *Wang Q. Liang X., Wang S.* Intra-islet glucagon secretion and action in the regulation of glucose homeostasis. Front. Physio., 2013, 3:485.doi: 10.3389/fphys.2012.00485.
25. *Wang Y., Li G., Goode J., Paz J.C. et al.* InsP3 Receptor Regulates Hepatic Gluconeogenesis in Fasting and Diabetes. Nature, 2012, v. 485(7396), p. 128–132.
26. *Yin H., Miao J., Zhang Y.* Protective effect of β-casomorphin-7 on type 1 diabetes rats induced with streptozotocin. Peptides, 2010, 31, p. 1725–1729.