https://doi.org/10.46389/rjd-2024-1776
www.rjdnmd.org

Rom J Diabetes Nutr Metab Dis
2024; volume 31, issue 4, pages 371–378

Original Article

Antidiabetic effect of a GABA-supporting mixture in a streptozotocin-induced diabetes model in rats

Narine Khachatryan[1], Rima Balagyozyan[1], Valentina Balagyozyan[1], Gayane Hekimyan[1], Gohar Grigoryan[1], Sona Mardanyan[1]*, Alvard Antonyan[1]

[1] Department of Metabolism of Adenylic Compounds, H. Buniatyan Institute of Biochemistry of Armenian NAS, Yerevan, Armenia

* Correspondence to: Sona Mardanyan, Department of Metabolism of Adenylic Compounds, H. Buniatyan Institute of Biochemistry of Armenian NAS, Paruir Sevak Str., 5/1, 0014, Yerevan, Armenia. Phone: +37410297343; E-mail: biochem@biochem.sci.am

Received: 7 September 2024 / Accepted: 29 November 2024

Abstract

Gamma-aminobutyric acid, GABA, can stimulate the growth and viability of pancreatic β-cells, normalizing blood glucose levels. Our study aimed to investigate the antidiabetic effects of a GABA-supporting mixture (GSM) containing asparagine, glutamine, β-alanine, and ethanolamine-O-sulfate in a streptozotocin-induced diabetes model in rats. The animals with diabetes were injected with GSM daily for 5 days, followed by controlling glycemic status and measuring the activity of enzymes involved in the synthesis of GABA, glutaminase (GLS), and glutamate decarboxylase (GAD). Glucose levels in healthy (Control), diabetes (STZ), and GSM treated (STZ-GSM) animals were 4.48±0.3, 23.09±2.3, and 12.94±2.03 mM/L, respectively. The GAD activity in the same three groups was in homogenates of: brain 783±23, 1022±34, 829±33 IU/mg; pancreas 793±34, 1079±55, 857±58 IU/mg; liver 643±33, 804±15, 690±35 IU/mg, respectively. The GLS activity in these groups was: in the blood plasma 26±3, 78±6, 46±5 IU/mg; in homogenates: brain 83±2, 71±3, 86±3 IU/mg; pancreas 150±20, 220±25, 170±22 IU/mg; liver 59±5, 52±6, 81±7 IU/mg, respectively. Increased liver GLS in the STZ-GSM group indicated GABA support by GSM through an increase in GABA precursor glutamine. Conclusion: GSM improves glycemic status, GLS, and GAD activity in STZ-diabetic animals, confirming its applicability in the treatment of diabetes.

Keywords: diabetes mellitus, glucose level, glutaminase, glutamate decarboxylase.

Introduction

Diabetes mellitus (DM) is an endocrine disease with sustained high blood sugar levels. The major types of diabetes are: insulin-dependent or type 1 diabetes (T1DM) and non-insulin-dependent or type 2 diabetes (T2DM). T1DM is characterized by a severe deficiency of insulin secretion from β-cells in the Langerhans islets (absolute insulin deficiency) and patients need constant, lifelong insulin treatment. The 90% of diabetes patients are type 2 diabetics. In this case, the problem is the resistance of peripheral tissues (and cells) to insulin. In the experimental study, such chemicals as streptozotocin (STZ), and alloxan damage β-cells in laboratory animals, leading to the development of DM models. Via GLUT2 transporters, these chemicals accumulate in β-cells as glucose analogs and exert cytotoxic effects causing diabetes through β-cell death [1–3]. The harmfulness of alloxan consists of the generation of superoxide anions both in the β-cells and extracellular environment. The absorbed by β-cells STZ is broken down to glucose and methyl nitro-urea and exhibits a cytotoxic effect on β-cells causing modification of biological macromolecules, fragmentation of DNA etc. [4].

γ-Aminobutyric acid (GABA) is a non-proteinogenic amino acid, the main inhibitory neurotransmitter in the central nervous system of humans and other mammals [3]. In addition to the nervous system, GABA is also synthesized in relatively high levels in the insulin-producing islet β-cells of the pancreatic islets and inhibits secreting from islet α-cells of insulin counteracting glucagon. GABA promotes the survival of

β-cells and the conversion of α-cells to β-cells [3, 5–7]. Moreover, GABA inhibits and attenuates immune activations and inflammation in DM which results in the regulation of glucose homeostasis and the reduction of diabetic complications. Therefore, GABA is considered one of the approaches in DM treatments [8, 9].

Currently, many drugs are used in the treatment of diabetes and its complications. However, most patients do not have satisfactory results, so the development of new medicines remains to be a biochemical and pharmacological task. There are several registered drugs composed of different amino acids and peptides [10]. Particularly, to create an antidiabetic preparation, Prof. Kamalyan compiled a GABA-supporting mixture, which included GABA, glutamine (Gln), β-alanine (Ala), and ethanolamine-O-sulphate (EOS). The study showed the promising therapeutic effects of this mixture on the function of the pancreas in the alloxan diabetes model in rats [11]. Ala, a homolog of GABA, is formed by β-decarboxylation of aspartic acid [12]. It is provided with neuroprotective effects, immune system activating, and antioxidant properties. The neuronal uptake and neuronal receptor sensitivity to Ala were demonstrated, suggesting that the compound may be a false transmitter replacing GABA [13]. Gln is the most abundant amino acid in the blood and participates in the synthesis of several amino acids, vitamin D3, glutathione, glucosamine, serotonin etc. It regulates blood insulin levels and stabilizes glucose [14]. Gln is deaminated by the enzyme glutaminase, forming Glu and releasing ammonia. This reaction is important in various metabolic pathways, including the regulation of nitrogen balance in the body. The resulting neurotransmitter Glu is decarboxylated by the enzyme glutamate decarboxylase to form GABA [15]. EOS is an inhibitor of the enzyme GABA transaminase that prevents the metabolism of GABA. It is usually used as a biochemical tool in the study of GABA-involved processes [16].

Figure 1 presents the classical (solid lines) and alternative (dotted lines) pathways of GABA formation from precursor amino acids, Gln and Glu.

Glutaminase (GLS, EC 3.5. 1.2), a regulator of glutaminolysis is abundant in the liver and pancreatic β-cells [17]. There are two tissue-specific isoforms of GLS, a kidney-type (GLS1, 70 kDa) and a liver-type (GLS2, 58 kDa). GLS1 is expressed in the brain, heart, kidney, muscle, intestine, lung, pancreas, and lymph node, and increases in response to metabolic acidosis. GLS2 is expressed in the liver and increases in starvation, diabetes, and high protein diet [18, 19].

Glutamate decarboxylase (GAD, EC 4.1.1.15) catalyzes the removal of the α-carboxyl group from L-Glu to produce GABA and CO_2. In humans, GAD is expressed in the brain and β cells of the pancreas as isoenzymes GAD65 and GAD67. GAD65 is one of the strongest autoantigens that triggers T-cell-mediated autoimmune diabetes in the human pancreas [20]. GAD and GABA are present in "GABA-ergic" nerve cells, but they are also detected in certain non-neural cells and organs particularly in the pancreas [21]. Both GABA and GAD are highly expressed in various islets of Langerhans demonstrating the importance of GABA in the biology of the pancreas [15].

Prof. Kamalyan proposed an alternative pathway for the synthesis of GABA from Gln, bypassing the formation of excitotoxic Glu. In this way, Gln is decarboxylated by isoenzyme GAD65 with the formation of

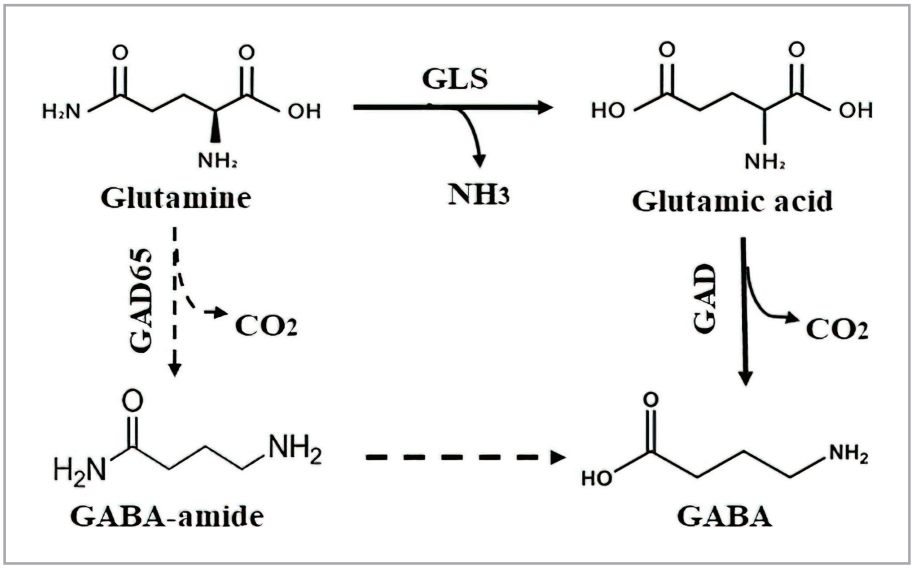

Figure 1: GABA synthesis pathways; dotted – alternative path proposed by Prof. Kamalyan.

https://doi.org/10.46389/rjd-2024-1776

GABA-amide, followed by its deamidation into GABA (Scheme 1) [22]. GABA-amide, which is probably a storage form of GABA, has been shown to exert an inhibitory effect on neurons in both the substantia nigra of the brain and interneurons in the spinal cord [23]. The presence of GABA-amide in the brain and its alternative generation from Gln provides additional flexibility to the amino acid transmitter system.

In the present research, we modified the offered by Prof. R.G. Kamalyan mixture to reveal some enzymatic mechanisms of the registered anti-diabetic effects of the constituents. The modified mixture included amino acid asparagine (Asn), a precursor of Gln [24].

The aim of this work was to investigate the antidiabetic effects of a GABA-supporting mixture (GSM) containing asparagine, glutamine, β-alanine, and ethanolamine-O-sulfate in an STZ-induced diabetes model in rats. The relative analysis of the activities of the GLS and GAD enzymes might expand the understanding of the pathogenesis of diabetes and offer new approaches to improve the processes compromised by the disease.

Material and methods

Chemicals

Pyridoxal 5'-phosphate (PLP) was purchased from MERCK (Germany), Streptozocin (STZ), γ-aminobutyric acid (GABA), β-alanine (Ala), ethanolamine-O-sulfate (EOS), Asparagine (Asp), Glutamine (Gln), Glutamic acid (Glu), bromocresol green, were purchased from Sigma-Aldrich (USA). Other reagents were of the highest purity available.

Objects

In the studies, 150-190g weight laboratory white rats are used. The animals are kept under normal nutritional conditions providing a 12-hour light regime, and at the ambient temperature of 22–24˚C. Experiments were conducted by a Guide for the Care and Use of Laboratory Animals by the European Parliament, Directive 2010/63/EU, and were approved by the Local Bioethical Committee.

Procedures

The materials of the research were blood plasma, pancreas, brain, and liver from rats. For experimental diabetes, the animals were divided into three groups of 7–10 rats each. The first group served as a control group. The animals of the other two groups were injected with streptozotocin (STZ) at a dose of 40 mg/kg of animal weight to develop a diabetes model. Animals were weighed and blood glucose level was monitored daily after night starvation using the glucometer. When the blood glucose level exceeded 15 mmol/l, the animals were considered diabetic. Half of the diabetic animals 5 days were daily injected with 0.5 ml/100g weight of physiological solution as the diabetic control group (STZ). The other part of diabetic animals 5 days were daily injected with 0.5 ml/100 g weight of freshly prepared GSM: 30mg Asn + 30mg Gln + 15mg Ala + 30 mg EOS in 3 ml water. The animals were anesthetized by administration of 40–50 mg/kg Nembutal, injected I.P., using a hypodermic needle, and were decapitated. Before decapitation, blood samples were collected from the jugular vein of rats under general anesthesia using a syringe with heparin as an anticoagulant and centrifuged at 6000 rpm for 10 minutes to separate plasma. The brain, pancreas, and liver were isolated, and washed in distilled water. All experimental samples were stored in a refrigerator at -20˚C until use.

Preparation of homogenates

Each of the brain, pancreas, and liver tissues were weighed and homogenized using a Potter-Elweheim homogenizer in 1 mM EDTA and 5 mM DTT containing K-Na-phosphate buffer (pH 8.4) at a ratio of 1:9 (weight/volume). The homogenates were centrifuged at 10.000 rpm for 20 minutes. The supernatants were kept in the refrigerator at – 20˚C until assay.

Enzyme assay

Glutaminase (GLS) activity was determined by the modified phenol-hypochlorite colorimetric method of Chaney and Marbach measuring the amount of liberated ammonia in the reaction of catalyzed enzymatic deamination of Gln [25]. The assay mixture in the volume of 0.4 ml contained 80 mM K-Na-phosphate buffer (pH 8.4) and a homogenate sample under study (20–100 μg protein). The enzymatic reaction was started by the addition of 0.1 ml 10 mM Gln and the mixture was incubated for 40 min at 37˚C. Then, per 1 ml each of phenol-nitroprusside and hypochlorite reagents were added and after 15 minutes incubation, the absorbance at 630 nm was registered. Ammonium sulfate solution was used as a standard. The enzyme activity was expressed in μmol of released ammonia per minute (IU) per mg of protein in the test mixture [26].

Glutamate decarboxylase (GAD) activity was evaluated by the modified colorimetric assay [27]. GAD catalyzes α-decarboxylation of L-glutamic acid (Glu) to GABA and needs pyridoxal 5'-phosphate (PLP) as a cofactor. The enzymatic decarboxylation of Glu proceeds with the release of CO_2 accompanied by the irreversible incorporation of a proton and GABA formation. The increase of pH due to the consumption of protons at the proceeding of the GAD-catalyzed reaction was detected. To record the change in pH, bromocresol green was utilized. Shortly, the 1980 µl reaction mixture contained 20 mM acetate buffer, pH 4.8, 70 µM bromocresol green, 10 mM PLP, and a sample under study (20–100 µg protein). The enzymatic reaction proceeded after the addition of 20 µl Glu from a 1 M stock solution in 20 mM acetate buffer, pH 4.8. The absorbance of the assay mixture was measured at 620 nm (A_0). Then, the mixture was incubated at 37°C for 20 min and the optical absorbance was recorded again at 620 nm (A_{20}). The enzymatic reaction rate is calculated from the dA/dt, where $dA=(A_{20}-A_0)$, dt=20 min. Applying the extinction coefficient for bromocresol green ε_{620} = 23.7 $mM^{-1}cm^{-1}$, the Glu protonation was evaluated as an enzymatic activity of GAD. It was expressed in IU per mg of protein in the assay mixture [27].

Protein concentration

Protein concentration was evaluated using the Coomassie Blue dye as described in [28].

Equipment

The spectral measurements were carried out at 25°C on the spectrophotometer Cary 60 (USA), using quartz cuvettes with optical path of 0.5 or 1.0 cm.

Statistics

All data were analyzed using the GraphPad Prism 8.0.1 (244). Specific differences are examined using A sample t-test and Ordinary one-way ANOVA (Multiple comparisons). The data showing p-values of <0.05 were considered statistically significant. Results are expressed as means±SEM.

Results

To studies experimental diabetes, the animals were divided into three groups of 7–10 rats in each: 1) Control, 2) STZ, 3) STZ+GSM. The glycaemic status was controlled and the activities of the GLS and GAD enzymes, participating in the synthesis of GABA, were measured in the serum, brain, liver, and pancreas of all three group animals.

Glucose level monitoring

In the animals of the control group, the mean blood glucose level measured after overnight starvation was 4.48±0.3 mM/L. In the animals of the STZ control group (40 mg/kg STZ-induced diabetes), the mean blood glucose level was 23.09±2.3 mM/L, which was statistically higher compared to the healthy group animals (p<0.0001) (Figure 2).

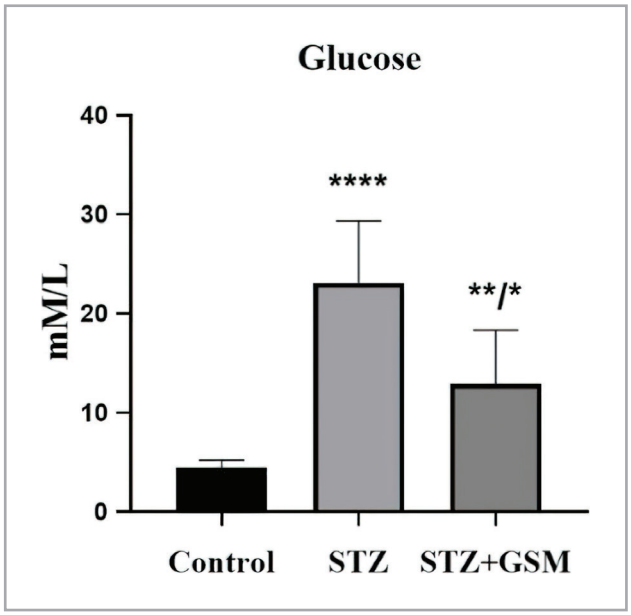

Figure 2: Blood glucose levels in the Control, STZ-induced diabetes animals (STZ) and the diabetic animals treated with the GSM (STZ+GSM), (mean±SEM, n=7), ****p<0.0001 *vs.* Control; **p=0.0024 *vs.* STZ; *p=0.01 *vs.* Control.

The present aimed to find out the therapeutic effect of GSM in animals with STZ-induced diabetes. For this purpose, a part of diabetic animals was injected with GMS (Asn/Gln/Ala/EOS in ratio 2/2/1/2, total concentration 35 mg/mL). Figure 2 demonstrates that a 5-day daily injection of 0.5 ml mixture per 100g animal weight lowered the blood glucose level in the STZ+GSM group animals down to 12.94±2.03 mM/L. This glucose level is of statistical significance lower compared with the animals in the diabetic, STZ group (p=0.0024), however, remaining to be higher than in the healthy animals of the Control group (p=0.01).

https://doi.org/10.46389/rjd-2024-1776

GLS Activity

It is believed that neuroactive GSM can promote the regeneration of damaged β-cells, and their proliferation, and enhance the synthesis and release of insulin [11, 24]. Figure 3 A shows the effect of the studied GSM on GLS activity in the blood plasma of rats. The 40 mg/kg STZ injection significantly increased not only blood glucose level but also GLS activity. It was statistically higher in blood plasma samples of diabetic rats compared with the Control (78±6 IU/mg of protein in animals of the STZ group and 26±3 IU/mg in animals of the Control group $p<0.0001$). A 5-day injection of the GSM led to the diminishing of the GLS activity down to 46±5 IU/mg, ($p=0.002$), while remaining statistically higher compared to healthy animals in the Control group ($p=0.038$).

GLS is one of the key enzymes regulating GABA levels in pancreatic cells, which is important for the functioning of islet cells in the synthesis and secretion of insulin [29]. Figure 3 B shows the effect of STZ-induced diabetes and the studied GSM on the activity of GLS in rat pancreatic homogenates. In the STZ group, a 1.5-fold increase in pancreatic GLS activity was recorded compared to the healthy Control group (220±20 and 150±20 IU/mg, respectively, $p=0.04$). A daily injection of the GSM for 5 days diminished GLS activity in the pancreas down to 170±20 IU/mg ($p=0.16$). This difference is not significant but is notable that the GLS activity in the STZ+GSM group became closer to the value in the

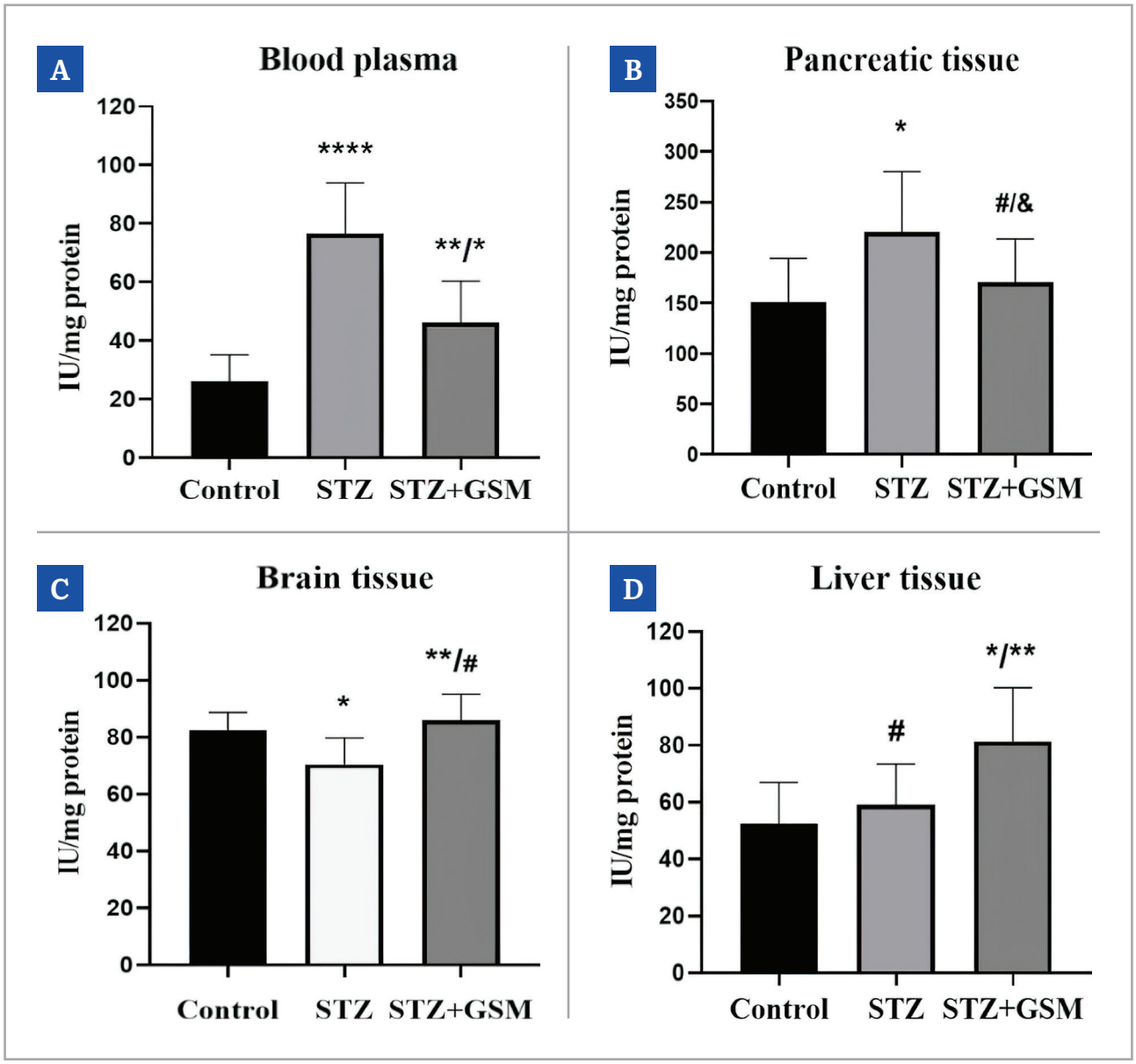

Figure 3: GLS activity in the blood plasma (A), pancreatic (B), brain (C), and liver (D) homogenates of rats in the Control, STZ, and STZ+GSM groups (M±SEM, n=7).

pancreatic homogenate of the Control group animals (p=0.73).

Figure 3 C shows the effect of GSM on GLS activity in rat's brain homogenates. The STZ administration decreased GLS activity in rat brain homogenate in a statistically significant manner from 83±2 IU/mg in the Control to 71±3 IU/mg in the STZ group (p=0.04). For 5 days, daily injection of GSM led to an increase in the GLS activity in the STZ+GSM group to 86±3 IU/mg, which is significantly higher than the activity in the STZ group (p=0.007), and closer to the value in the brain homogenate of animals in the Control group (p=0.72).

Figure 3 D shows the effect of diabetes induction and GSM on the activity of GLS in rat liver homogenates. In this case, injection of STZ resulted in a slight increase in the activity of GLS in rat liver homogenates from 52±6 IU/mg in Control to 59±5 IU/mg in STZ group (p=0.72). A 5-day injection of the GSM resulted in a 1.37-fold increase in GLS activity in the STZ+GSM group up to 81±7 IU/mg (p=0.046). This GLS activity is 1.6-fold higher than in the Control group (p=0.009).

Glutamate Decarboxylase (GAD) Activity

The equilibrium between L-Glu and GABA is regulated by the enzyme GAD which catalyzes the removal of the α-carboxyl group from L-Glu to produce GABA and CO_2. Inhibition/activation of GAD activity both *in vitro* and *in vivo* is under close attention by a number of researchers [30]. A five-day injection of the studied GSM led to a decrease in enzyme activity by 18.8% in the brain, 14.14% in the liver, and 20.5% in the pancreas (Table 1).

Discussion

GABA is a non-proteinogenic amino acid and neurotransmitter, which is produced in the islets at levels comparable to those in the brain. It is synthesized by the enzyme GAD65, the major autoantigen in type 1 diabetes. When released, GABA influences the activity of multiple islet cell types through its receptors. Being an inhibitory neurotransmitter in the central nervous system of humans, GABA is also known for its antidiabetic activity. However, while the function of GABA in the nervous system is well understood, the role of the islet GABA system is unclear due to dissimilarities in describing reports [15]. The present study aims to contribute to the elucidation of the mechanism of participation of the islet GABA system in the pathology of diabetes and the possibility of improving the course of the disease using this system.

Islet GABA levels are reduced in both type 1 and type 2 diabetes. The reason for this reduction is unknown, but the deficiency of islet GABA correlated with the pathogenesis of diabetes and led to alpha, beta, and delta cell dysfunction. Understanding the role of GABA in the pathology of diabetes may explain how GABA contributes to the islet dysfunction that accompanies all forms of diabetes.

GABA is known to be useful in treating diabetes, because it prevents the destruction, stimulates the proliferation and regulates the mass of pancreatic β-cells, and promotes the synthesis and release of insulin [3]. Earlier, Prof. R.G. Kamalyan proposed and successfully tested the mixture, containing GABA, Ala, and Gln, as well as the EOS to prevent the metabolism of GABA. This mixture showed an antidiabetic effect in the alloxan diabetes model in rats [11].

To elucidate the biochemical mechanisms contributing to the GABA-associated antidiabetic effects, the present study tested a GABA-supporting mixture (GSM) consisting of Asn, Gln, Ala, and EOS. The activity of the GLS and GAD enzymes involved in GABA synthesis was compared in tissue homogenates of the brain, pancreas, and liver of rats with STZ-induced diabetes, with and without the injection of GSM. It was found that the development of diabetes mellitus leads to a significant increase in the activity of the GABA-synthesizing enzyme GAD in homogenates of all studied tissues compared to those in healthy animals. A 5-day injection of the studied GSM resulted in a significant decrease in GAD activity in all homogenates, shifting the increased

Table 1: GAD activity (IU per mg protein) in organ homogenates of animals in the Control, STZ, and STZ+GSM groups (M±SEM, n=7).

Tissue (n=7)	Control	STZ	p *vs.* control	STZ+GSM	p *vs.* control	p *vs.* STZ
Brain	783±23	1022±34	***p=0.0001	829±33	p=0.56	**p=0.001
Liver	643±33	804±15	**p=0.003	690±35	p=0.49	*p=0.034
Pancreas	793±34	1079±55	**p=0.002	857±58	p=0.64	*p=0.016

https://doi.org/10.46389/rjd-2024-1776

values closer to the control ones (Table 1). Thus, the last reaction of the classical pathway of GABA synthesis from Glu (Figure 1) was impaired due to the development of STZ-induced diabetes but is normalized by the object of our study, GSM.

Glu, a precursor for the biological synthesis of GABA, is formed in the GLS-catalyzed reaction of Gln catabolism with the release of ammonia (Scheme 1) [31]. The activity of GLS increased significantly in the pancreatic homogenate of animals with diabetes ($p=0.04$ *vs*. Control). The injection of GSM reduced it to a level noticeably closer to the control value ($p=0.73$) (Figure 3 B). Our study showed a significant decrease in GLS activity in diabetic rat brain homogenate. A 5-day injection of GSM brought this parameter closer to control values (Figure 3 C). Hence, in the brain and pancreas GSM injection normalizes GLS activity shifted by the development of diabetes.

In contrast to these organs, GLS activity in the liver increased only slightly in diabetic animals, confirming the results reported by Vijay with colleagues and Squires with colleagues [31, 32]. Surprisingly, GSM injection significantly increased GLS levels compared to both control and STZ cases (Figure 3 D). This observation can probably be interpreted as a promotion by GSM in the contribution of GLS to the enhancement of GABA by increasing its precursor Glu in the liver.

We saw that the presence of GSM brought the studied activities of GABA synthesis enzymes closer to the norm. Probably, the alternative pathway proposed by Prof. Kamalyan also works in the case of our GSM, when Gln is synthesized from the added Asn, and then GABA-amide is formed from Gln, resulting in the synthesis of GABA (Scheme 1, dotted paths). Besides, the injection of a mixture containing the GABA transaminase inhibitor EOS, which interferes with GABA metabolism, together with the GABA homolog Ala, presumably increases the concentration of GABA in the rat tissues, providing a decrease in blood glucose levels.

Conclusion

Based on the results of the research, we can conclude that the studied GSM along with a significant hypoglycaemic effect, is capable of restoring the activity of GLS and GAD, reduced in animals with diabetes caused by STZ. Considering that increased GABA levels are known to be beneficial in diabetes treatment, we hypothesize that the new GABA-support mixture may serve as an additional approach in the treatment of DM. Further studies are needed with longer-term monitoring and possibly varying the ratios of the mixture components.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

The study was approved by the Ethics Committee of the Institute of Biochemistry of the National Academy of Sciences of the Republic of Armenia; International Registrations' Numbers IRB0001621; IORG 0009782.

Funding

This work was carried out within the framework of the 23AN: NS - biochem-2895 grant of the Fund for Armenian Relief.

References

1. Lenzen S. The mechanisms of alloxan and streptozotocin-induced diabetes. Diabetologia. 51(2):216-226, 2008.
2. Lenzen S. Alloxan and streptozotoin diabetes. Advanced Research Institutes of Diabetes Animals. 6(4):113-122, 2010.
3. Purwana I, Zheng J, Li X, et al. GABA promotes human β-cell proliferation and modulates glucose homeostasis. Diabetes. 63(12):4197–205, 2014.
4. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res.50(6):537-46, 2001.
5. Soltani N, Qiu H, Aleksic M, et al. GABA exerts protective and regenerative effects on islet beta cells and reverses diabetes. Proc Natl Acad Sci.108(28):11692–1197, 2011. doi:10.1073/pnas.1102715108.
6. Tian J, Dang H, Chen Z, Guan A, Jin Y, Atkinson MA, et al. γ-Aminobutyric acid regulates both the survival and replication of human β-cells Atkinson. Diabetes. 62(11):3760–3765, 2013. doi:10.2337/db13-0931.
7. Korol SV, Jin Z, Jin Y, et al. Functional characterization of native, high-affinity GABAA receptors in human pancreatic β cells. EBioMedicine. 30:273–82, 2018.
8. Mendu SK, Bhandage A, Jin Z, et al. Different subtypes of GABA-A receptors are expressed in human, mouse, and rat T lymphocytes. PLoS One.7(8):e42959, 2012.
9. Ben-Othman N, Vieira A, Courtney M, et al. Long-Term GABA Administration Induces Alpha Cell-Mediated Beta-like Cell Neogenesis. Cell. 168(1–2):73–85, 2017. doi: 10.1016/j.cell.2016.11.002.
10. Ding B, Zhu Zh, Guo C, et al. Oral peptide therapeutics for diabetes treatment: State-of-the-art and future perspectives. Acta Pharmaceutica Sinica B.14(5):2006-2025, 2024.

11. Khachatryan RS, Khachatryan NKh, Kamalyan RG. Antidiabetic effect of a complex of neuroactive amino acids in an alloxan diabetes model. Biological Journal of Armenia.4(71):66-69, 2019.
12. Danielle SM, Struys EA, Hogema BM, et al. Development of a stable-isotope dilution assay for γ-aminobutyric acid (GABA) transaminase in isolated leucocytes and evidence that GABA and β-alanine transaminases are identical. Clinical Chemistry.47:525–531, 2001.
13. Gladkevich A, Korf J, Hakobyan VP, et al. The peripheral GABAergic system as a target in endocrine disorders. Auton Neurosci.124(1-2):1-8, 2006. doi: 10.1016/j.autneu.2005.11.002.
14. Cruzat V, Macedo Rogero M, Noel Keane K, et al. Glutamine: Metabolism and Immune Function, Supplementation and Clinical Translation. Nutrients.10(11):1564, 2018.
15. Hagan DW, Ferreira SM, Santos GJ, et al. The role of GABA in islet function. Front Endocrinol (Lausanne). 13:972115, 2022. doi: 10.3389/fendo.2022.972115.
16. Starr MS, Tanner T. Effects of Ethanolamine-O-sulfate and GABA on the contents of GABA and various amines in brain slices. Neurochem.25:573-577, 1975.
17. Deguchi-Horiuchi H, Suzuki S, Lee EY. Pancreatic β-cell glutaminase 2 maintains glucose homeostasis under the condition of hyperglycemia. Sci Rep.13:7291, 2023. https://doi.org/10.1038/s41598-023-34336-z.
18. Krause Mda S, de Bittencourt PI Jr. Type 1 diabetes: can exercise impair the autoimmune event. The L-arginine/glutamine coupling hypothesis. Cell Biochem Funct.26(4):406-33, 2008.
19. Lu Ding, Xiaonan Xu, Congcong Li, et al. Glutaminase in microglia: A novel regulator of neuroinflammation. Brain, Behavior and Immunity.92:139-156, 2021. https://doi.org/10.1016/j.bbi.2020.11.038.
20. Kash SF, Condie BG, Baekkeskov S. Glutamate decarboxylase and GABA in pancreatic islets: lessons from knock-out mice. Horm Metab Res.31(5):340-344,1999.
21. Jun HS, Khil LY, Yoon JW. Role of glutamic acid decarboxylase in the pathogenesis of type 1 diabetes. Cell Mol Life Sci.59:1892–1901, 2002.
22. Kamalyan RG, Vardanyan AG. About the exchange of GABA and GABA amides in the brain. Neurochemistry.29(2):118-121, 2012.
23. Sarkisyan DS, Kamalyan RG, Galoyan AA, et al. Isolated and combined effect of GABA, GABA-amide, glutamine and ethanolamine-O-sulfate on the electrical activity of single neurons in the central. Neurochemistry. 24(1):41-51, 2007.
24. Khachatryan N Kh. Pathways of amide metabolism in the pancreas. Biol. Journal of Armenia, 71(2):62-65, 2019.
25. Chaney A, Marbach E. Modified reagents for determination of urea and ammonia, Clin Chem. 8:130-132, 1962.
26. Mardanyan S, Sharoyan S, Antonyan A, et al. Tryptophan Environment in Adenosine Deaminase: Enzyme Modification with N-bromosuccinimide in the Presence of Adenosine and EHNA Analogs. Biochem. Biophys. Acta. 1546:185–195, 2001. https://doi.org/10.1016/s0167-4838(01)00141-8.
27. Yu K, Hu S, Huang J, et al. A high-throughput colorimetric assay to measure the activity of glutamate decarboxylase. Enzyme Microb Technol. 49(3):272-276, 2011.
28. Bradford MM. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Anal Biochem.72:248-254,1976.
29. Suzuki S, Tanaka T, Poyurovsky MV, et al. Phosphate-activated glutaminase (GLS2), a p53-inducible regulator of glutamine metabolism and reactive oxygen species. Proc Natl Acad Sci USA.107(16):7461-7466, 2010. doi: 10.1073/pnas.1002459107.
30. Genčić MS, Stojanović NM, Mladenović MZ, et al. An HPLC-based assay for improved measurement of glutamate decarboxylase inhibition/activation. Neurochem Int. 161:105433, 2022. doi: 10.1016/j.neuint.2022.105433.
31. Vijay V, Kashinath RT, Sandhya HP, et al. Glutaminase activity in various tissues and their relative organ weights in long term (90 days) Alloxan Diabetic Rats. Medica Innovatica. 3(1):180-186, 2014.
32. Squires SA, Ewart HS, McCarthy C, et al. Regulation of Hepatic Glutaminase in the Streptozotocin-Induced Diabetic Rat. Diabetes 1. 46(12):1945-1949, 1997.

https://doi.org/10.46389/rjd-2024-1776