IMPACT OF MELATONIN ON SOME INDICATORS OF CARBOHYDRATE METABOLISM IN LIVER OF RATS WITH DEXAMETHASONE-INDUCED DIABETES

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One frequently employed experimental model, particularly in pre-clinical studies examining the hypoglycemic effects of potential antidiabetic medications, is the metasteroid diabetes model induced by the prolonged administration of glucocorticoids to animals. This experimental study aimed to elucidate the effects of exogenous melatonin (10 mg/kg) on glycogen content and the activity of key enzymes—pyruvate kinase (PK) [EC 2.7.1.40], lactate dehydrogenase (LDH) [EC 1.1.1.27], glucose-6-phosphate dehydrogenase (G-6-PDH) [EC 1.1.1.49], and glucose-6-phosphatase (G-6-P-ase) [EC 3.1.3.9]—in the livers of rats with dexamethasone-induced diabetes. Materials and Methods. The experiments were performed on 44 male 18-month-old white non-linear rats, divided into three groups: 1) control (intact rats), 2) rats with dexamethasone-induced diabetes, 3) rats that amid the progression of dexamethasone-induced diabetes, underwent daily oral administration of melatonin (Sigma, USA) in a dose of 10 mg/kg. Dexamethasone diabetes was modeled by subcutaneous injection of dexamethasone (injection solution 4 mg/ml, KRKA, Slovenia) at a dose of 0.125 mg/kg body weight daily for 13 days (O.V. Stefanov, 2001). Decapitation of animals was carried out in accordance with the norms of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986). Glucose content in blood from the tail vein of rats, taken on the 14th day of fasting before decapitation of the animals, was determined using a portable glucometer (One Touch Ultra Easy, Life Scan, USA). The content of glycogen and the activity of the studied enzymes of carbohydrate metabolism in the liver were determined according to the generally accepted, previously described methods. A 5% homogenate was prepared from the cold-isolated rat liver in a chilled 50 mM Tris-HCl buffer (pH=7.4) to study the activities of pyruvate kinase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase and glucose-6-phosphatase in the cytosolic fraction. The reliability of the difference between the obtained indicators was assessed using the parametric Student's t-test (for normal distribution) and the non-parametric Mann-Whitney U-test (for non-normal distribution). Differences were considered probable at p≤0.05. Results and discussion. According to our results, in the liver of diabetic rats that did not receive any means of correction of carbohydrate metabolism disorders, the glycogen content was 33% lower than in intact animals. The activities of enzymes such as pyruvate kinase and glucose-6-phosphate dehydrogenase were also reduced in the liver of rats with impaired glucose tolerance by 31.6 and 21.5%, respectively, compared to intact animals, indicating inhibition of glucose oxidation pathways, both at the level glycolysis (decrease of pyruvate kinase), as well as at the level of the oxidative stage of the pentose-phosphate pathway of glucose-6-phosphate oxidation. At the same time, the activity of lactate dehydrogenase and especially glucose-6-phosphatase in the liver of rats with diabetes by 19.5 and 56%, respectively, exceeded the indicators of animals of the control group, which demonstrates the increased activity, intensity of glycogenolysis and gluconeogenesis under conditions of insulin resistance, because glucose-6-phosphatase is the terminal enzyme of these processes. Regarding the investigated parameters of carbohydrate metabolism, both the glucose content in the blood of rats, as well as the glycogen content and the activity of all studied enzymes in the liver of rats that were injected with melatonin against the background of the development of diabetes, did not reliably differ from the parameters of intact animals, which confirms the assumption of the probable hypoglycemic effect of melatonin against the background of the development of diabetes. Conclusions: The daily two-week use of melatonin (10 mg/kg), against the background of the development of dexamethasone diabetes in rats, contributes to the normalization of certain indicators of carbohydrate metabolism in the liver of animals.

Key words: carbohydrate metabolism, liver, dexamethasone diabetes, melatonin, rats.

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Introduction

Diabetes mellitus, despite significant advances in its treatment is still remaining as the most common endocrine disease of mankind that leads to loss of working capacity and disability, and sometimes even to the death [6].

According to the World Health Organization experts, diabetes is a state of chronic hyperglycemia caused by an impairment of the insulin synthesis or cell response to insulin. Diabetes mellitus is a chronic endocrine-metabolic disease caused by an absolute and/or relative lack of insulin that is caused by the impact of various endogenous (genetically determined) and exogenous factors. This leads to the disruption of various metabolic processes, particularly carbohydrate metabolism, resulting in damage caused by hyperglycemia and the subsequent glycosylation of various organs and tissues [5].

In the early years of the second millennium, specifically in 2000, the global number of patients with diabetes mellitus stood at 175.4 million. By 2015, this figure had risen to 245 million [8], and projections indicate a substantial escalation to 642
million by the year 2040. Notably, the majority of individuals with diabetes are diagnosed with type 2 diabetes, which is characterized by significant insulin resistance. Type 2 diabetes mellitus is a consequence of a disturbance in insulin homeostasis, ranging from a predominance of insulin resistance with a relative insufficiency to a predominance of a secretion disorder, with or without insulin resistance [17]. This type is primarily marked by the development of peripheral tissue resistance to insulin and dysfunction of the pancreatic beta cells. These factors contribute to the emergence of pathological conditions such as glucose toxicity and lipotoxicity, ultimately culminating in the onset of vascular complications that pose a significant threat to the body [8].

Hence, addressing the challenge of identifying novel, effective methods for diagnosing and pharmacologically correcting diabetes and its associated complications remains a pressing issue today. Endocrinologists worldwide are actively engaged in the intensive exploration of solutions to this problem. The ongoing search for innovative approaches and the use of new medications that would demonstrate both high therapeutic efficacy and safety for the body are of paramount relevance.

Currently, researchers have at their disposal more than ten diverse experimental models capable of replicating the key pathogenetic elements of diabetes either in vitro or in vivo. One frequently employed experimental model, particularly in pre-clinical studies examining the hypoglycemic effects of potential antidiabetic medications, is the metamasteroid diabetes model induced by the prolonged administration of glucocorticoids to animals [6, 8].

Melatonin (N-acetyl-5-methoxytryptamine), primarily synthesized by the pineal gland, serves not only as a synchronizer of circadian rhythms and a regulator of sleep but also as a key player in glucose homeostasis regulation. Exogenous melatonin is currently under consideration as a regulator of sleep but also as a key player in the emergence of pathological consequences of a disturbance in insulin homeostasis, ranging from a predominance of insulin resistance with a relative insufficiency to a predominance of a secretion disorder, with or without insulin resistance [17]. This type is primarily marked by the development of peripheral tissue resistance to insulin and dysfunction of the pancreatic beta cells. These factors contribute to the emergence of pathological conditions such as glucose toxicity and lipotoxicity, ultimately culminating in the onset of vascular complications that pose a significant threat to the body [8].

Thus, investigating the impact of melatonin on glycogen content and the activity of liver enzymes, which are indicators of carbohydrate metabolism during diabetes development, is pertinent.

This experimental study aimed to elucidate the effects of exogenous melatonin (10 mg/kg) on glycogen content and the activity of key enzymes—pyruvate kinase (PK) [EC 2.7.1.40], lactate dehydrogenase (LDH) [EC 1.1.1.27], glucose-6-phosphate dehydrogenase (G-6-PDH) [EC 1.1.1.49], and glucose-6-phosphatase (G-6-P-ase) [EC 3.1.3.9]—in the livers of rats with dexamethasone-induced diabetes.

Materials and Methods. The experiments were performed on 44 male 18-month-old white non-linear rats, divided into three groups: 1) control (intact rats), 2) rats with dexamethasone-induced diabetes, 3) rats that amid the progression of dexamethasone-induced diabetes, underwent daily oral administration of melatonin (Sigma, USA) in a dose of 10 mg/kg.

Dexamethasone–induced diabetes was modeled by subcutaneous injection of dexamethasone (injection solution 4 mg/ml, KRKA, Slovenia) in a dose of 0.125 mg/kg body weight daily for 13 days (O.V. Stefanov, 2001). Insulin resistance of experimental rats was assessed using the Homeostasis Model Assessment (HOMA) mathematical model (D.R. Matthews et al., 1985). Glucose content in blood from the tail vein of rats was determined on an empty stomach on the 14th day immediately before the decapitation of the animals using a portable glucometer (One Touch Ultra Easy, Life Scan, USA); the content of insulin in the blood serum of rats was determined using the "Maglumi" test kit (China) using an automatic immunochemiluminescence analyzer (Snibe Co., Ltd, China). The index of insulin resistance (HOMA-IR) was calculated according to the formula: HOMA-IR=(glucose (mmol/l)•insulin (μU/ml))/22.5. Decapitation of animals was carried out in accordance with the norms of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

Glucose content in blood from the tail vein of rats, taken on the 14th day of fasting before decapitation of the animals, was determined using a portable glucometer (One Touch Ultra Easy, Life Scan, USA).

The content of glycogen and the activity of the studied enzymes of carbohydrate metabolism in the liver were assessed according to the generally accepted, previously described methods [17]. A 5% homogenate was prepared from the cold-isolated rat liver in 50 mM Tris-HCl buffer (pH=7.4) to study the activities of PK, LDH, G-6-PDH and G-6-P-ase in the cytosolic fraction [4, 16].

The reliability of the difference between the obtained indicators was assessed using the parametric Student's t-test (for normal distribution) and the non-parametric Mann-Whitney U-test (for non-normal distribution). Differences were considered probable at p≤0.05.

Results. Two-week daily administration of dexamethasone solution in the above-mentioned dose to 18-month-old rats causes the development of resistance of peripheral tissue receptors to the action of insulin with subsequent increase in the concentration of glucose in the blood (fig. 1), changes that are characteristic of type 2 diabetes.
In animals subjected to a 14-day course of dexamethasone injections, a disturbance in glucose tolerance arises. This model effectively replicates crucial pathogenetic mechanisms underlying the development of diabetes, specifically impairments in the action of the hormone insulin, characteristic of individuals with type 2 diabetes.

Dexamethasone-induced diabetes is known as one of the models for the study of hypoglycemic agents [7, 8], since the administration of excessive doses of glucocorticoids leads to a violation of the secretory function of the beta cells of the islets of Langerhans of the pancreas and the development of insulin resistance. In experimental animals with dexamethasone-induced diabetes, there is a deterioration in the action of insulin.

The mechanisms of the development of pathological processes induced by dexamethasone have not been thoroughly studied, but it is known that dexamethasone increases the production of islet amyloid polypeptide - amylin, which is synthesized by pancreatic beta cells and is the main component of islet amyloid, which is formed in patients with type 2 diabetes. Amylin is known to have a suppressive effect on insulin action in tissues. Inhibition of insulin secretion in combination with amyloidogenic properties suggest a possible role of amylin in the pathogenesis of type 2 diabetes. Dexamethasone injections also reduce glucose utilization by adipocytes by directly affecting the expression of glucose transporters GLUT 1 and GLUT 4, leading to the development of insulin resistance. At the same time, the inhibitory effect of glucocorticoids on the secretory activity of pancreatic beta cells is possibly related to the inactivation of mitochondrial FAD-glycerophosphate dehydrogenase, an enzyme that plays a key role in glucose-induced insulin secretion [2, 3].

The formation of diabetes in experimental animals is confirmed by an increase in the level of glucose in the blood. In the blood of all diabetic rats on the 14th day, the glucose content exceeded 8.9 mmol/l (fig. 1).

One of the main pathogenetic mechanisms of impaired carbohydrate metabolism in diabetes is the slowing down of the hexokinase reaction in the tissues, and the glucokinase reaction of glucose-6-phosphate formation in the liver, which subsequently leads to the development of an ATP energy deficit in them [10, 11].

In the liver of diabetic rats that did not receive correction of carbohydrate metabolism disorders, according to our results (table 2), the glycogen content was 33% lower than in intact animals.

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<table>
<thead>
<tr>
<th>Indicators</th>
<th>Glycogen content (mg%)</th>
<th>PK, micromol/min×mg</th>
<th>LDH, nmol/min×mg</th>
<th>G-6-PDH, nmol/min×mg</th>
<th>G-6-P-ase, microg/min×mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>3412.5±65</td>
<td>53.5±3.45</td>
<td>2.9±0.43</td>
<td>6.5±0.36</td>
<td>29.7±2.02</td>
</tr>
<tr>
<td>2. Diabetes</td>
<td>2289.4±57*</td>
<td>36.4±2.93*</td>
<td>3.6±0.52*</td>
<td>5.1±0.26*</td>
<td>67.4±3.27*</td>
</tr>
<tr>
<td>3. Diabetes + melatonin</td>
<td>3258.4±72*</td>
<td>51.2±4.19*</td>
<td>3.0±0.37*</td>
<td>6.1±0.44*</td>
<td>35.9±3.22*</td>
</tr>
</tbody>
</table>
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Note: 1. a, b - changes are reliable (p≤0.05). 2. a - concerning intact rats; 3. b - concerning diabetic rats

The activities of enzymes such as PK and G-6-PDH were also reduced in the liver of rats with impaired glucose tolerance by 31.6 and 21.5%, respectively, compared to intact animals, indicating inhibition of glucose oxidation pathways, both at the level of glycolysis (decrease of PK), as well as at the level of the oxidative stage of the pentose-phosphate pathway of glucose-6-phosphate
oxidation. At the same time, the activity of LDH and especially G-6-P-ase in the liver of rats with diabetes by 19.5 and 56%, respectively, exceeded the indicators of animals of the control group, which demonstrates the increased intensity of glycogenolysis and gluconeogenesis under conditions of insulin resistance, because glucose-6-phosphatase is the terminal enzyme of these processes. Melatonin injections helped to normalize all investigated indicators.

Discussion. With absolute or relative insulin deficiency, as is known [9] the processes of glucose entering tissues are disrupted, the formation of glucose-6-phosphate decreases, the intensity of glycogen synthesis in tissues decreases, the process of glycolysis and aerobic oxidation of glucose is inhibited. Glycogen synthase, as is known, is active in dephosphorylated form under the action of insulin, and its lack leads to a decrease in the intensity of glycogenesis. In the liver, the deficiency of glucose-6-phosphate in diabetes is known to be compensated by its formation through gluconeogenesis from non-carbohydrates, in particular from amino acids and lactate. Absolute or relative insulin deficiency leads to suppression of the intensity of aerobic conversion of pyruvate as a result of inhibition of pyruvate dehydrogenase, and therefore of oxidative decarboxylation of pyruvate to acetyl-CoA. At the same time, pyruvic acid is reduced to lactic under the action of lactate dehydrogenase (mainly LDH 5 or 4 in the liver), and thanks to glucocorticoids and glucagon, pyruvate carboxylase is activated, which catalyzes the formation of oxaloacetate, which is mostly directed to the formation of glucose through gluconeogenesis.

Activation of gluconeogenesis in the liver of diabetic rats is to some extent explained by the predominance of glucocorticoids, which, against the background of tissue resistance to insulin, increase the synthesis of enzymes in the liver that channel bypass reactions of gluconeogenesis, including glucose-6-phosphatase. It is the increase in the activity of hepatic glucose-6-phosphatase that is considered a significant cause of the development of diabetic hyperglycemia and related complications, in particular due to intensive glycosylation of a number of proteins. It is also known that the development of insulin deficiency is accompanied by a decrease in the activity of such a bifunctional enzyme as phosphofructokinase-2/fructose-2,6-bisphosphatase [EC 2.7.1.105/3.1.3.46], and it depends on the content of fructose-2,6-bisphosphate in the liver cells the intensity of both glycolysis and gluconeogenesis, because it allosterically activates phosphofructokinase (glycolysis) and inhibits fructose-1,6-bisphosphatase (gluconeogenesis). In diabetes, the activity of fructose-1,6-bisphosphatase and glucose-6-phosphatase is increased [13, 14].

Regarding the examined parameters of carbohydrate metabolism, including blood glucose levels, glycogen content, and the activity of all studied enzymes in the livers of rats administered melatonin during diabetes development, no statistically significant differences were observed compared to the parameters in intact animals. This finding supports the hypothesis of a potential hypoglycemic effect of melatonin in the context of diabetes development [1, 17].

Therefore, the administration of melatonin (10 mg/kg) to rats in parallel with dexamethasone prevents the development of insulin resistance in animals, the increase of hyperglycemia and the development of carbohydrate metabolism disorders, in particular, it contributes to the normalization of the glycogen content in the liver and the activities of certain enzymes that characterize glucose metabolism - PK (glycolysis); LDH (glycolysis and gluconeogenesis); G-6-PDH (pentose phosphate pathway of glucose-6-phosphate oxidation) and G-6-P-ase (glycogenolysis and gluconeogenesis).

Conclusions.

The daily two-week administration of melatonin (10 mg/kg) during the progression of dexamethasone-induced diabetes in rats leads to the normalization of specific indicators of carbohydrate metabolism in the animals’ livers.

References.

ВПЛИВ МЕЛАТОНІНУ НА ДЕЯКІ ПОКАЗНИКИ ОБМІНУ ВУГЛЕВОДІВ У ПЕЧІНЦІ ЩУРІВ ПРИ ДЕКСАМЕТАЗОНОВОМУ ДІАБЕТІ

Яремій І.М., Кушнер О.Ю., Яремій К.М.

Ключові слова: обмін вуглеводів, печінка, дексаметазоновий діабет, мелатонін, щурі.

Серед однієї із поширенних експериментальних моделей, яку використовують для дослідження гіпо-глікемійної дії потенційних антидіабетичних препаратів на етапі до клінічних спостережень є модель так званого метастероїдного цукрового діабету, який викликає шляхом тривалого введення тваринам глікогонікіїдних субстанцій.

Мета дослідження. Вивчити вплив екзогенного мелатоніну (10 мг/кг) на вміст глікогену й активності піруваткінази [КФ 2.7.1.4], лактатдегідрогенази [КФ 1.1.1.27], глюкозо-6-фосфатдегідрогенази [КФ 1.1.1.49] та глікозо-6-фосфата [КФ 3.1.3.9] в печінці щурів із дексаметазоновим діабетом.

Матеріали та методи. Експерименти проведено на 44 самцях 18-місячних білих нелінійних щурів, яких було поділено на три групи: 1) контрольна (інтактні щурі), 2) щурі з дексаметазоновим діабетом, 3) щурі, яким на фоні розвитку дексаметазонідукованого діабету щоденно перорально вводили мелатонін (Sigma, США) в дозі 10 мг/кг. Дексаметазоновий діабет моделювали шляхом підшкірного введення тваринам дексаметазону (розчин для ін'єкцій 4 мг/мл, КRKA, Словенія) в дозі 0,125 мг/кг маси тіла щоденно впродовж 13-ти діб (О.В. Стефанов, 2001). Декапітацію тварин проводили згідно норм «Європейської конвенції по захисту хребетних тварин, які використовуються в експериментальних та інших наукових цілях» (Страсбург, 1986).

Щодо досліджуваних показників обміну вуглеводів, то як уміст глюкози в крові щурів, так і вміст глікогену був на 33% нижчим, ніж у інтактних тварин. Активність всіх досліджуваних ферментів у печінці щурів, яких на фоні розвитку діабету вводили мелатонін не відрізнявся від показників інтактних тварин, що підтверджує припущення про ймовірну гіпо-глікемійну дію мелатоніну на фоні розвитку діабету.

Висновок: Щоденне двотижневе застосування мелатоніну (10 мг/кг), на фоні розвитку в щурів дексаметазонового діабету, сприяє нормалізуванню окремих показників обміну вуглеводів у печінці тварин.

Відомо, що у співвідношенні з різноманітними фетальними шкодами, ввічливістю засобами корекції порушень обміну вуглеводів, вміст глікогену був на 33% нижчим, ніж у інтактних тварин. Активність всіх досліджуваних ферментів у печінці щурів, яких на фоні розвитку діабету вводили мелатонін не відрізнявся від показників інтактних тварин, що підтверджує припущення про ймовірну гіпо-глікемійну дію мелатоніну на фоні розвитку діабету.

Висновок: Щоденне двотижневе застосування мелатоніну (10 мг/кг), на фоні розвитку в щурів дексаметазонового діабету, сприяє нормалізуванню окремих показників обміну вуглеводів у печінці тварин.