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THE EFFECT OF MELATONIN ON THE ENERGY METABOLISM IN HEART OF DIABETIC RATS

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The aim of this study was to determine the effect of melatonin on activity of pyruvate kinase (PK) and lactate dehydrogenase (LDH) in the heart tissue and on the activity of succinate dehydrogenase (SDG) and H+-ATPase in mitochondria of cardiomyocytes of rats with dexamethasone diabetes. Methods. The research was performed in compliance with the Rules of the work with test animals (1977) and the European Convention on the Protection of Vertebrate Animals used in experiments and other scientific purposes (18 March 1986). The experiments were carried out on 36 sexually mature male albino rats with the body mass 0.18 – 0.20 kg. The animals were divided into 3 groups:1) control group; 2) group with diabetes mellitus DM (BG level ≥ 8.0 mmol/l); 3) diabetic animals which were injected with melatonin. Melatonin ("Sigma", USA) was injected intraperitoneally in a dose of 10 mg/kg of body weight daily over 13 days. Dexamethasone diabetes was evoked by daily subcutaneous administration of dexamethasone (solution for injection 4 mg / ml, KRKA, Slovenia) at a dose of 0.125 mg / kg body weight for 13 days (O.V. Stefanov, 2001). The mathematical model of the insulin-glucose correlation by the Homeostasis Model Assessment (HOMA) was used to evaluate the insulin resistance in the animals (D.R.Matthews et al., 1985). The blood was taken from the tail vein to evaluate the BG level using "OneTouchUltra" ("LifeScan", USA). The rats were sacrificed on the 14^{th} day of the experiments in accordance with the ethical treatment of animals. 5% homogenate was prepared from cold-isolated rat hearts on chilled 50 mM Tris-HCl buffer (pH = 7.4) with sucrose. Mitochondria were isolated by differential centrifugation. Enzyme activities in the mitochondrial fraction was determined by: succinate dehydrogenase (SDG) according to the method of Eshchenko N.D. et al. (1992), H + -ATP-ase - for Gabibiv M.M. (1986). Serum insulin content was determined using an automatic immunochemiluminescent analyzer (Snibe Co., Ltd, PRC) using a Maglumi test kit. The insulin resistance index (HOMA-IR) was calculated by the formula: HOMA-IR = (glucose (mmol / l) • insulin (mcU / ml)) / 22.5. To determine the enzymes activities by standart methods, heart muscle tissue was quickly removed, rinsed in saline, blotted, weighed and homogenized. Then the homogenate (5% in ice-cold 0.25 mM tris-HCl-buffer) was ultracentrifugated (10 min at 1500r/min) and the supernatant was used for measurements. Statistical analysis was performed using Statistica 10 (StatSoft Inc). Prior to analysis, Shapiro-Wilk test was used to assess the normality of the group data. According to the criterion, the samples distributions differed from normal distribution. Given these, use of the Mann-Whitney test was considered sufficient for valid conclusions to be made. Differences were considered to be statistically significant when $P \leq 0.05$. Results. In the blood of all diabetic rats on the 14th day the glucose content exceeded 8.9 mmol / l, and the HOMA-IR index increased 7 times. According to results, we have obtained the activity of PK in the heart tissue of diabetic animals was 55% less than in control group. It can be explained by low uptake of glucose from the blood by heart muscle tissue in conditions of insulin resistance. Reduced glucose uptake by peripheral tissues, in turn, leads to a reduced rate of glucose metabolism. That means now substrate for next catabolic changes in glycolysis. The activity of LDH increases by 32% in the heart muscles of diabetic rats compared with the control values. We have found out that the activity of PK and LDH has become normalized by the introducing of melatonin in dose of 10 mg/kg of body weight. In the mitochondria of the animals with dexamethasone diabetes, the activity of SDG and H^+ -ATPase is significantly lower than that in intact animals by 45 and 32%, respectively. Probably, melatonin enhances the uptake of glucose by heart muscles. The blood glucose content of diabetic rats, which were administered is not significantly different from that of the intact animals, and the HOMA-IR was 6.3 times lower than that of diabetic rats. The activity of SDG and H^+-ATPase in the mitochondria of rats receiving both melatonin and dexamethasone does not differ from that of intact rats. Conclusion. A 14-day introduction of melatonin leads to a marked improvement of the state of carbohydrate metabolism as well as the energy metabolism in heart tissue that is accompanied by the normalization of the indices studied.

Key words: mitochondria, dexamethasone diabetes, heart, melatonin, rats.

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Introduction

Diabetes mellitus (DM) can damage eyes, kidneys, nerves and heart [1]. Microvascular and macrovascular disorders are the leading causes of morbidity and mortality in diabetic patients [13]. Hyperglycemia can result in increase in the indicators of lipid peroxidation and oxidative stress, in which free radicals play the main role in the pathogenesis of these complications [11].

Circadian disruption and obesity predispose to the Type 2 diabetes (T2DM) development, signifying that therapeutic targeting of both circadian and metabolic dysfunctions should be considered as a potential treatment approach. Importantly, the combination of melatonin and metformin produces synergistic action by modifying progression of metabolic dysfunction in circadian disruption obesity in rats through the improvement

of adiposity, circadian activity, insulin sensitivity and islet cell failure. At the same time, melatonin therapy alone improves circadian activity rhythms, attenuates induction of beta cell failure and enhances glucose tolerance [9].

It has been known that changes in the light-dark cycle in vivo entrain the phase of islet clock transcriptional oscillations, whereas prolonged exposure (10 weeks) to light at night disrupts islet circadian clock function through impairment in the amplitude, phase, and inter islet synchrony of clock transcriptional oscillations. Also, it has been reported that light at night exposure leads to diminished glucose-stimulated insulin secretion due to a decrease in insulin secretory pulse mass. This study identifies potential mechanisms by which disturbances in circadian rhythms, quite typical for modern life, can predispose to the islet failure in T2DM [3]. The clock disruption leads to transcriptome-wide alterations in the expression of islet genes involved in growth, survival and synaptic vesicle assembly. Notably, conditional ablation of the pancreatic clock causes diabetes mellitus (DM) due to impaired beta-cell function at the very latest stage of stimulus-secretion coupling. These results demonstrate a role of the beta-cell clock in coordinating insulin secretion with the sleep-wake cycle and reveal that ablation of the pancreatic clock can trigger the DM onset [4].

It was shown that 14-day introduction of melatonin to alloxan diabetic rats under conditions of constant darkness led to a decrease in the basal glycaemia level as well as to the stabilization in the indices of the antioxidant defense disturbed by an absolute deficit of insulin [8].

Melatonin (N-acetyl-5-methoxytryptamine) is the major product of the pineal gland, which functions as a regulator of sleep, circadian rhythm, and immune function [15]. Melatonin and its metabolites have potent antioxidant/anti-inflammatory properties, and they have proven to be highly effective in a variety of disorders associated to inflammation and oxidative stress [14].

Dexamethasone diabetes enables designing the most important pathogenetic mechanisms for the development of diabetes types two [7].

The aim of this study was to determine the effect of melatonin on activity of pyruvate kinase [EC 2.7.1.40] (PK) and lactate dehydrogenase [EC 1.1.1.27] (LDH) in heart tissue and on the activity of succinate dehydrogenase [EC 1.3.5.1.] (SDG) and H^+-ATPase [EC 3.6.3.6] in mitochondria of cardiomyocytes in rats with dexamethasone diabetes.

Materials and methods

The research was performed in compliance with the Rules of the work with experimental animals (1977) and the European Convention on the Protection of Vertebrate Animals used in experiments and other scientific purposes (18 March 1986). The experiments were carried out on 63 sexually mature male albino rats with the body mass of 0.18 – 0.20 kg. The animals were divided into 3 groups: 1) control group; 2) group with DM (BG level ≥ 8.0 mmol/l); 3) diabetic animals, which were injected with melatonin. Melatonin (“Sigma”, USA) was injected intraperitoneally in a dose of 10 mg/kg of body weight daily over 13 days. Dexamethasone diabetes was evoked by daily subcutaneous administration of dexamethasone (solution for injection 4 mg / ml, KRKA, Slovenia) in a dose of 0.125 mg / kg body weight for 13 days (O.V. Stefanov, 2001) [7]. The mathematical model of the insulin-glucose linkage of the Homeostasis Model Assessment (HOMA) was used to evaluate the insulin resistance of the animals (D.R.Matthews et al., 1985). The blood was taken from the tail vein to evaluate the BG level using “OneTouchUltra” (“LifeScan”, USA). The rats were sacrificed at the 14th day of the experiments in accordance with the ethical treatment of animals. 5% homogenate was prepared from cold-isolated rat hearts on chilled 50 mM Tris-HCl buffer (pH = 7.4) with sucrose. Mitochondria were isolated by differential centrifugation. Enzyme activity in the mitochondrial fraction was determined by succinate dehydrogenase (SDG) according to the method of Eshchenko N.D. et al. (1992), H^+-ATP-ase by Gabibiv M.M. (1986). Serum insulin content was assessed by using an automatic immunochemiluminescent analyzer (Snibe Co., Ltd, PRC), a Maglumi test kit. The insulin resistance index (HOMA-IR) was calculated by the formula: HOMA-IR = (glucose (mmol / l) • insulin (mcU / ml)) / 22.5. To determine the activity of PK and LDG by standard methods [9], heart muscle tissue was quickly removed, rinsed in saline, blotted, weighed and homogenized. Then the homogenate (5% in ice-cold 0.25 mM tris-HCl-buffer) was ultracentrifugated (10 min at 1500r/min) and the supernatant was used for measurements.

Statistical analysis was performed using Statistica 10 (StatSoft Inc). Prior to analysis, Shapiro-Wilk test was used to assess the normality of the group data. According to the criterion, the samples distributions differed from normal distribution. The use of the Mann-Whitney test was considered sufficient for valid conclusions to be made. Differences were considered to be statistically significant when $P \leq 0.05$

Results

On the 14th day glucose content in the blood of all diabetic rats exceeded 8.9 mmol / l, and the HOMA-IR index increased 7 times. According to results obtained, the activity of PK in heart tissue of diabetic animals was by 55% less than in the control group.

Table 1

Changes inf the carbohydrate metabolism in the heart of diabetic rats, (n=6, x±S$\bar{x}$)

Indices / Groups	PK, mkmol/min×mg	LDG, nmol/min×mg
1.Control group	40,2±1,32	2,5±0,09
2. DM	14,5±1,07[a]	3,4±0,28[a]
3. DM + melatonin	43,3±1,19	2,8±0,03[b]

Note: 1. a, b, c - changes are reliable (p≤0,05). 2. a - concerning intact rats; b - concerning rats with diabetes mellitus.

It can be explained by low uptake of glucose by the cardiac muscular tissue from the blood t in conditions of insulin resistance [5]. Reduced glucose uptake by peripheral tissues in turn leads to a reduced rate of glucose metabolism. That means now substrate for next catabolic changes in glycolysis [3]. In our opinion, less sensitivity to insulin resulted in blockage of pyruvate dehydrogenase complex that causes the increase in LDH activity on 32% in the heart muscles of the diabetic rats compared with the control values. We have found out that the activity of PK and LDH become normalized by the introducing melatonin in a dose of 10 mg/kg of body weight.

Probably, melatonin enhances the uptake of glucose by heart muscles [6].

The blood glucose content of rats, which were given melatonin against the background of diabetes was not significantly different from that in intact animals, and the HOMA-IR was 6.3 times lower than that in diabetic rats.

SDG is the only enzyme that participates in both the citric acid cycle and the electron transport chain. ATPases in mitochondria are the prime producers of ATP, and use the proton gradient generated by oxidative phosphorylation [2].

In the mitochondria of animals with dexamethasone diabetes, the activity (tab.1) of SDG and H^+ -ATPase were significantly lower than that of intact animals by 45 and 32%, respectively.

Table 2

*Changes of mitochondrial energy function in heart of diabetic rats, **(n=6, x±S$\bar{x}$)***

Indexes / Groups	SDH, nmol/min×mg	H^+-ATP-ase, mkmol (iP)/min×mg
1.Control group	12,74±1,651	0,54±0,028
2. DM	7,08±0,593[a]	0,35±0,021[a]
3. DM + melatonin	11,25±1,97[b]	0,52±0,044[b]

Note: 1. a, b, c - changes are reliable (p≤0,05). 2. a - concerning intact rats; b - concerning rats with diabetes mellitus.

The activity of SDG and H^+-ATPase in the mitochondria of rats receiving both melatonin and dexamethasone did not differ from that in intact rats.

Oxidative stress plays a pivotal role in the development of diabetes complications, both microvascular and cardiovascular. Melatonin [4, 12], a potent antioxidant agent, is essential for glucose homeostasis and regulation. It has been determined (Czuczejko J., Sielski Ł., 2019) that melatonin supplementation influenses oxidative stress parameters in elderly patients with non-insulin dependent diabetes mellitus.

Moreover, earlier [8, 9,10] we investigated Langergans islets in diabetic rats and recorded histomorphological alterations: their pancreatic share reliably decreased by 55%, number and percentage of beta-cells with necrosis decreased by 90% and 97% respectively compared with the control. Melatonin treatment led to a sharp decrease in the elevated serum glucose and partial regeneration/proliferation of beta-cells. It was concluded that the hypoglycemic action of melatonin could be partly due to amelioration in beta-cells of pancreatic islets.

Melatonin stimulates glucose transport to skeletal muscle cells via insulin receptor of substrate-1/phosphoinositide-3-kinase pathway that implies, at the molecular level, its role in glucose homeostasis and, possibly, in diabetes. Endogenous melatonin level may contribute to the incidence and/or development of diabetes. A strong phosphorylation of inositol-requiring enzyme 1 (IRE-1), c-JUN NH2-terminal kinase (JNK) and insulin receptor substrate 1 (IRS-1) serine as well as a dramatic decrease in IRS-1 tyrosine phosphorylation were observed in the presence of tunicamycin. All of these led to a blockage of insulin signaling in skeletal muscle cells that was due to melatonin pretreatment. Melatonin may also increase a plasma concentration of leptin in mice [13] and there are findings that terminally ill insulin-deficient rodents with uncontrolled diabetes due to autoimmune or chemical destruction of beta-cells were made hyperleptinemic by an adenoviral transfer of the leptin gene. Within approximately 10 days their severe hyperglycemia and ketosis were corrected. Despite the lack of insulin, moribund animals resumed linear growth. Normoglycemia persisted for 10-80 days without other treatment, while normal physiological conditions lasted for approximately 175 days despite reoccurance of moderate hyperglycemia. Inhibition of gluconeogenesis by suppression of hyperglucagonemia and reduction of hepatic cAMP response element-binding protein, phoshoenolpyruvate carboxykinase and peroxisome proliferator-activated receptor-gamma-coactivator-1alpha may explain the anticatabolic effect. Up-regulation of insulin-like growth factor 1

(IGF-1) expression, plasma levels and increasing IGF-1 receptor phosphorylation in muscles may explain the increased insulin receptor substrate 1, PI3K and ERK phosphorylation in skeletal muscles. These findings suggest that leptin reverses the catabolic consequences of a total lack of insulin, potentially by suppressing glucagon action on the liver and enhancing the insulinomimetic actions of IGF-1 on skeletal muscle, and suggest strategies for making type 1 diabetes insulin-independent.

Conclusion

A 14-day introduction of melatonin leads to a marked improvement of the state of carbohydrate metabolism as well as the energy turnover in heart tissue accompanied by a normalization of the indices under study.

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Реферат

ВПЛИВ МЕЛАТОНІНУ НА ЕНЕРГЕТИЧНИЙ МЕТАБОЛІЗМ В СЕРЦІ ДІАБЕТИЧНИХ ЩУРІВ

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Ключові слова: мітохондрії, дексаметазоновий діабет, серце, мелатонін, щурі.

Метою було вивчити вплив мелатоніну на активності сукцинатдегідрогенази й H^+-АТФ-ази в мітохондріях серця, а також піруваткінази і лактатдегідрогенази (ЛДГ) в серцевому м'язі щурів із дексаметазоновим діабетом. Матеріали та методи. Експерименти проведено на самцях 18-місячних білих нелінійних щурів, яких було поділено на три групи: 1) інтактні, 2) щурі з дексаметазоновим діабетом, 3) щурі, яким на фоні розвитку діабету щоденно перорально вводили мелатонін (Sigma, США) в дозі 10 мг/кг. Дексаметазоновий діабет моделювали шляхом щоденного підшкірного введення тваринам дексаметазону (розчин для ін'єкцій 4 мг/мл, KRKA, Словенія) в дозі 0,125 мг/кг маси тіла впродовж 13-ти діб (О.В. Стефанов, 2001). Для оцінювання інсулінорезистентності тварин використовували математичну модель інсулін-глюкозного зв'язку Homeostasis Model Assessment (HOMA) (D.R.Matthews et al., 1985). Уміст глюкози в крові з хвостової вени щурів, взятої на 14-ту добу натще перед декапітацією тварин, визначали за допомогою портативного глюкометра (One Touch Ultra Easy, Life Scan США). Уміст інсуліну в сироватці крові визначали за допомогою автоматичного імунохемілюмінісцентного аналізатора (Snibe Co., Ltd, КНР) з використанням тест-набору "Maglumi", КНР. Індекс інсулінорезистентності (HOMA-IR) розраховували за формулою: HOMA-IR=(глюкоза (ммоль/л)•інсулін (мкОд/мл))/22,5. Декапітацію тварин проводили згідно норм «Європейської конвенції по захисту хребетних тварин, які використовуються в експериментальних та інших наукових цілях» (Страсбург, 1986). З ізольованого на холоді серця щурів готували 5% гомогенат на охолодженому 50мМ Трис-HCI-буфері (pH=7,4) із сахарозою. Мітохондрії виділяли методом диференційного центрифугування. Активності ферментів у мітохондріальній фракції визначали: сукцинатдегідрогеназу – згідно методики Eshchenko N.D. et al. (1992), H^+-АТФ-ази – за Gabibiv M.M. (1986). Активності піруваткінази та ЛДГ визначали за стандартними методиками. Достовірність різниці між отриманими показниками оцінювали з використанням параметричного t-критерію Ст'юдента (при нормальному розподілі) та непараметричного U-критерію Манна-Уїтні (при невідповідності нормальному розподілу). Відмінності вважали вірогідними при $p \le 0,05$. Результати. Дексаметазоновий діабет, як відомо (О.В. Стефанов, 2001), є однією з моделей для дослідження гіпоглікемійних засобів, оскільки введення надмірних доз глюкокортикоїдів призводить до порушення секреторної функції панкреатичних бета-клітин і розвитку інсулінорезистентності. У крові всіх діабетичних щурів на 14-ту добу вміст глюкози перевищив 8,9 ммоль/л, а індекс

HOMA-IR збільшився в 7 разів. У мітохондріях тварин із дексаметазоновим діабетом активності сукцинатдегідрогенази і H^+-АТФ-ази, а також активність піруваткінази у тканині серця – були вірогідно нижчими, ніж у інтактних тварин – на 45 і 32, та 55% відповідно. Активність лактатдегідрогенази у діабетичних тварин зросла на 32% порівняно з контролем. Уміст глюкози в крові щурів, яким на фоні розвитку діабету вводили мелатонін, не відрізнявся вірогідно від показників інтактних тварин, а показник HOMA-IR був у 6,3 разів нижчим, ніж у діабетичних щурів. Активності сукцинатдегідрогенази і H^+-АТФ-ази в мітохондріях щурів, а також піруваткінази і лактатдегідрогенази в тканині серця, які одночасно отримували мелатонін і дексаметазон не відрізнялися від показників інтактних щурів. Висновки. Щоденне двотижневе застосування мелатоніну, на фоні розвитку в щурів дексаметазонового діабету, сприяє нормалізуванню енергетичного обміну в серці тварин, шляхом підвищення активностей мітохондріальної сукцинатдегідрогенази й H^+-АТФ-ази та піруваткінази на фоні зниження активності лактатдегідрогенази.

Реферат

ВЛИЯНИЕ МЕЛАТОНИНА НА ЭНЕРГЕТИЧЕСКИЙ МЕТАБОЛИЗМ В СЕРДЦЕ ДИАБЕТИЧЕСКОЙ КРЫС
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Ключевые слова: митохондрии, дексаметазоновый диабет, сердце, мелатонин, крысы.

Целью было изучить влияние мелатонина на активности сукцинатдегидрогеназы и H^+-АТФазы в митохондриях сердца, а также пируваткиназы и лактатдегидрогеназы в сердечной мышце крыс с дексаметазоновым диабетом. Материалы и методы. Эксперименты проведены на самцах 18-месячных белых нелинейных крыс, которые были разделены на три группы: 1) интактные, 2) крысы с дексаметазоновым диабетом, 3) крысы, которым фоне развития диабета ежедневно перорально вводили мелатонин (Sigma, США) в дозе 10 мг/кг. Дексаметазоновый диабет моделировали путем ежедневного подкожного введения животным дексаметазона (раствор для инъекций 4 мг/мл, KRKA, Словения) в дозе 0,125 мг / кг массы тела в течение 13-ти суток (А. Стефанов, 2001). Для оценки инсулинорезистентности животных использовали математическую модель инсулин-глюкозной связи Homeostasis Model Assessment (HOMA) (D.R.Matthews et al., 1985). Содержание глюкозы в крови из хвостовой вены крыс, взятой на 14-е сутки натощак перед декапитацией животных, определяли с помощью портативного глюкометра (One Touch Ultra Easy, Life Scan США). Содержание инсулина в сыворотке крови определяли с помощью автоматического имунохемилюминисцентного анализатора (Snibe Co., Ltd, КНР) с использованием тест-набора "Maglumi", КНР. Индекс инсулинорезистентности (HOMA-IR) рассчитывали по формуле: HOMA-IR = (глюкоза (ммоль / л) • инсулин (МКОД / мл)) / 22,5. Декапитацию животных проводили в соответствии с нормами «Европейской конвенции по защите позвоночных животных, используемых в экспериментальных и других научных целях» (Страсбург, 1986). С изолированного на холоде сердца крыс готовили 5% гомогенат на охлажденном 50 мМ Трис-HCl-буфере (pH=7,4) с сахарозой. Митохондрии выделяли методом дифференциального центрифугирования. Активности ферментов в митохондриальной фракции определяли: сукцинатдегидрогеназу - согласно методике Eshchenko N.D. et al. (1992), H^+-АТФазы - по Gabibiv M.M. (1986). Активности пируваткиназы и лактатдегидрогеназы определяли по стандартным методикам. Достоверность различий между полученными показателями оценивали с использованием параметрического t-критерия Стьюдента (при нормальном распределении) и непараметрического U-критерия Манна-Уитни (при несоответствии нормальному распределению). Различия считали достоверными при $p \leq 0,05$. Результаты. Дексаметазоновый диабет, как известно (А. Стефанов, 2001), является одной из моделей для исследования гипогликемийных средств, поскольку введение чрезмерных доз глюкокортикоидов приводит к нарушению секреторной функции панкреатических бета-клеток и развития инсулинорезистентности. В крови всех диабетических крыс на 14-е сутки содержание глюкозы превысил 8,9 ммоль / л, а индекс HOMA-IR увеличился в 7 раз. В митохондриях животных с дексаметазоновым диабетом активности сукцинатдегидрогеназы и H^+-АТФазы, а также активность пируваткиназы в ткани сердца – были достоверно ниже, чем у интактных животных - на 45 и 32, и 55% соответственно. Активность лактатдегидрогеназы в диабетических животных выросла на 32% по сравнению с контролем. Содержание глюкозы в крови крыс, которым на фоне развития диабета вводили мелатонин не отличался достоверно от показателей интактных животных, а показатель HOMA-IR был в 6,3 раз ниже, чем у диабетических крыс. Активности сукцинатдегидрогеназы и H^+-АТФазы в митохондриях кардиомиоцитов крыс, а также активности пируваткиназы и лактатдегидрогеназы в ткани сердца крыс, которые одновременно получали мелатонин и дексаметазон не отличались от показателей контроля. Выводы. Ежедневное двухнедельное применение мелатонина, на фоне развития у крыс дексаметазонового диабета, способствует нормализации энергетического обмена в сердце животных, путем повышения активностей митохондриальной сукцинатдегидрогеназы и H^+-АТФазы и пируваткиназы на фоне снижения активности лактатдегидрогеназы.