

DYNAMICS OF DAILY RHYTHM CHANGES OF PANCREAS HORMONAL ACTIVITY IN LONG-TERM HYPOPINEALISM

L. Bondarenko¹, T. Mishchenko^{1,2,3}¹SI "V. Danilevsky Institute for Endocrine Pathology Problems National Academy of Medical Sciences of Ukraine", Kharkiv,²Kharkiv Institute of Medicine and Biomedical Sciences of PHEE "Kyiv Medical University"³V.N. Karazin Kharkiv National University.

Key words:
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E-mail:mishchenko2707
@ukr.net

Objective: to investigate features of insulinemia and glycemia chrono rhythms changes in long-term hypopinealism in dynamics.

Material and methods: the study was conducted on 15 male rabbits aged four and five months. Control animals were kept under the standard vivarium conditions and experimental animals were exposed to twenty-four hours lighting for ten months. Serum glucose and insulin concentrations were estimated by glucose oxidase and immunoassay methods, respectively.

Results: prolonged melatonin deficiency over the metabolic syndrome course causes levelling of circadian rhythm of insulin concentration in blood as a consequence of hypoinsulinemia during the day and absence of daily changes of circulatory glucose level due to hyperglycemia within twenty-four hours.

Conclusions: established changes of carbohydrate metabolism indicators should be regarded as a prerequisite for manifestation of type 2 diabetes mellitus hereafter.

Ключові слова:
гіпопінєалізм,
метаболический
синдром,
мелатонін,
інсулін, глюкоза.

Клінічна та
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ДИНАМІКА ЗМІН ДОБОВОГО РИТМУ ГОРМОНАЛЬНОЇ АКТИВНОСТІ ПІДШЛУНКОВОЇ ЗАЛОЗИ ПРИ ДОВГОТРИВАЛОМУ ГІПОПІНЕАЛІЗМІ

Л. Бондаренко, Т. Міщенко

Мета роботи: дослідити в динаміці особливості змін хроноритмів інсулінемії та глікемії при довготривалому гіпопінєалізмі.

Матеріали та методи: дослідження виконано на 15 самцях кроля віком чотири-п'ять місяців. Контрольних тварин утримували в стандартних умовах віварію, піддослідних - в умовах цілодобового освітлення впродовж десяти місяців. Концентрацію глюкози та інсуліну в сироватці крові визначали глюкозооксидазним та імуноферментним методами, відповідно.

Результати: тривала мелатонінова недостатність внаслідок перебігу метаболічного синдрому викликає нівелювання циркадного ритму концентрації інсуліну в крові в результаті гіпоінсулінемії протягом доби та відсутність добових змін циркуляторного рівня глюкози внаслідок сталої гіперглікемії.

Висновки. Виявлені зміни показників вуглеводного обміну слід розглядати як передумову для маніфестації цукрового діабету 2 типу в подальшому.

Ключевые слова:
гипопинеализм,
метаболический
синдром,
мелатонин,
инсулин, глюкоза.

Клиническая и
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ДИНАМИКА ИЗМЕНЕНИЙ СУТОЧНОГО РИТМА ГОРМОНАЛЬНОЙ АКТИВНОСТИ ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ ПРИ ДЛИТЕЛЬНОМ ГИПОПИНЕАЛИЗМЕ

Л. Бондаренко, Т. Мищенко

Цель работы: исследовать в динамике особенности изменений хроноритмов инсулинемии и гликемии при длительном гипопинеализме.

Материалы и методы: исследование выполнено на 15 самцах кроликов в возрасте четыре-пять месяцев. Контрольных животных содержали в стандартных условиях вивария, подопытных - в условиях круглосуточного освещения в течение десяти месяцев. Концентрацию глюкозы и инсулина в сыворотке крови определяли глюкозооксидазным и иммуноферментным методами, соответственно.

Результаты: длительная мелатониновая недостаточность при течении метаболического синдрома вызывает нивелирование циркадного ритма концентрации инсулина в крови в результате гипoinsулинемии на протяжении суток и отсутствие суточных изменений циркуляторного уровня глюкозы вследствие устойчивой гипергликемии.

Выводы. Установленные изменения показателей углеводного обмена следует рассматривать как предпосылку для манифестации сахарного диабета 2-го типа в дальнейшем.

Introduction

Currently the results of numerous chronobiological studies definitely show that the presence of behavior and physiological functions daily rhythms in most mammals is ensured by endogenous circadian clock [1]. As it is known one of the important conditions for its proper functioning is melatonin production by pineal gland, melatonin is involved in modulating some physiological systems (in particular immune and endocrine ones), metabolism and higher brain activity [2].

In our previous studies it has been found that keeping rabbits under the conditions of around the clock lighting (ACL) for five months causes the signs of the pineal gland, thyroid gland and gonads premature aging as well as metabolic syndrome (MS) development [3, 4]. Carrying out further chronobiological researches in this area provided evidence that formation of the main components of the symptom complex (insulin resistance, abdominal obesity, dyslipidemia and arterial hypertension) due to melatonin deficiency is associated with the daily rhythm disorders of their indexes [5, 6, 7, 8].

Objective

To investigate in dynamics features of insulinemia and glycemia chrono rhythms changes in long-term hypopinealism.

Material and methods

The study was conducted on 15 male rabbits aged four and five months (young mature animals) obtained from vivarium of SI" V. Danilevsky Institute for Endocrine Pathology Problems NAMS of Ukraine". In accordance with the experiment design animals were divided into two groups: 1) a control one ($n = 5$), which included the rabbits kept under the standard vivarium conditions; 2) an experimental one ($n = 10$), where the animals were in the conditions of ACL (during the day - natural sun light, at night - electric lighting with intensity in animal cages 30-40 lux) for ten months. Light intensity was measured by using luxmeter "U-117". Incandescent lamp with the power of 100 W was used as an artificial light source.

Chronobiological studies were based on two determinative points: day (13:00 - 14:00) and night (01:00 am - 2:00 pm). During these hours insulin and glucose concentration in rabbits' serum was determined monthly with the aim to establish their daily rhythms.

Glucose level in animals' serum was determined by glucose oxidase method using the enzymatic analyzer "Eksan-G" (Lithuania). Insulin concentration in rabbits' serum was determined by an immunoassay method using kits Insulin ELISA (DRG Instruments GmbH, Germany) and immunoassay analyzer Stat Fax 3200 ("Awareness", USA). For the kit the cross-over reaction of antibodies to human insulin with rabbit's insulin is 63%, herewith the amino acid sequence in human insulin molecule differs from that one in rabbits only by a variation in one position of the B-chain [9].

All procedures during the experimental study were carried out with animals in accordance with the methodological recommendations of the Ministry of

Health of Ukraine and the general ethical principles of conducting animal experiments agreed with the provisions of the "European Convention on the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes".

Statistical analysis of digital material was carried out using parametric and nonparametric methods. The verification of conformity of the data distribution to the Gauss law was carried out using the Shapiro-Wilk criterion. Comparing the groups with normal data distribution was carried out using the odd Student's t -criterion. The Mann-Whitney U -criterion was used to analyze the data distribution of which did not conform to the normal law. The results are presented in the form of $\bar{x} \pm s_{\bar{x}}$ (arithmetic mean (\bar{x}), standard error of the arithmetic mean ($s_{\bar{x}}$)). The significance level (p) was taken as $p \leq 0.05$ for determining the differences between the groups as being statistically significant.

Results and discussion

In studying the insulinemia daily rhythm in rabbits with MS against a background of long-term hypopinealism induced by ACL for ten months, the absence of circadian rhythm of this parameter in the experimental animals was established similarly as it was detected after keeping the rabbits under conditions of the disruption of the natural light/dark cycle for five months (Table 1).

However, the absolute indexes of insulinemia registered at this observation period have shown a rapid decrease in the concentration of this hormone in the experimental animals' circulation during the day as well as at night compared with the corresponding indexes determined after five months of ACL. It should be noted that the findings point to reversion of nocturnal hyperinsulinemia which was determined in experimental animals after five months ACL. Moreover, taking into account the established decrease level of this hormone in the animals' blood circulation during the day and night time after ten months of ACL relative to corresponding control parameters ($p < 0.05$) it becomes possible to affirm that the course of MS against a background of long-term hypopinealism is associated with the hypoinsulinemia formation throughout the day.

It has been established that the course of MS in rabbits against the background of long-term hypopinealism induced by ten months of ACL is associated with the preservation (namely, leveling) of glycemia daily rhythm disturbance determined during the formation of this symptom complex in the experimental animals as a result of ACL for five months (Table 2).

It should be noted that increase of ACL duration up to ten months caused an increase of blood glucose in rabbits in the day ($p < 0.001$) compared to an appropriate indicator determined in experimental animals after influencing the five months' ACL. Moreover, it has been found that in ten months of ACL glycemia in rabbits exceeds the control values both in the daytime and at night ($p < 0.001$ in both cases). In this connection, changes in circadian rhythm of blood glucose levels in experimental animals under the conditions of ten months' disruption of the natural light/

Table 1

Dynamics of daily rhythm changes of circulatory insulin level in rabbits with hypopinealism induced by around the clock lighting for ten months, pmol/l

| Group, the number of animals | Observation period, months | Statistical index | The time of day | |
|------------------------------|----------------------------|--|----------------------------------|-----------------------------------|
| | | | day | night |
| I (n = 11) | 0 (starting state) | $\bar{x} \pm S_{\bar{x}}$ | 105.37 ± 14.46 | 62.84 ± 7.49* |
| II (n = 6) | 5 | $\bar{x} \pm S_{\bar{x}}$ p_{I-II} | 131.10 ± 15.93 > 0.05 | 133.10 ± 16.30 < 0.001 |
| III (n = 5) | 10 | $\bar{x} \pm S_{\bar{x}}$ p_{I-III} p_{II-III} | 50.97 ± 2.84 < 0.05 < 0.05 | 45.22 ± 2.93 < 0.05 < 0.001 |

Note: * is a statistical significance in comparison with the indicator in the daytime, ($p < 0.05$)

Table 2

Dynamics of daily rhythm changes of circulatory glucose level in rabbits with hypopinealism induced by around the clock lighting for ten months, mmol/l

| Group, the number of animals | Observation period, months | Statistical index | Time of day | |
|------------------------------|----------------------------|--|-----------------------------------|----------------------------------|
| | | | day | night |
| I (n = 15) | 0 (starting state) | $\bar{x} \pm S_{\bar{x}}$ | 5.54 ± 0.20 | 3.92 ± 0.31* |
| II (n = 6) | 5 | $\bar{x} \pm S_{\bar{x}}$ p_{I-II} | 5.56 ± 0.45 > 0.05 | 6.50 ± 0.24 < 0.001 |
| III (n = 10) | 10 | $\bar{x} \pm S_{\bar{x}}$ p_{I-III} p_{II-III} | 6.39 ± 0.16 < 0.001 < 0.001 | 6.32 ± 0.20 < 0.001 > 0.05 |

Note: * is a statistical significance in comparison with the indicator in the daytime, ($p < 0.001$)

dark cycle are determined by hyperglycemia during the day, and not by the increase of this indicator only at night as it takes place in case of ACL over five months.

The obtained data can be explained by preliminary results of morphological and morphometric studies of the endocrine part of rabbits' pancreas under the condition of MS formation against the background of hypopinealism induced by ACL for five months. In particular, it has been shown that MS pathogenesis in hypopinealism is associated with the progressive loss of Langerhans' islets and their devastation as a result of a marked decrease in the number of β -cells against a background of the cellular structure degradation of the insulin-producing apparatus, as well as the functional overload of β -cells with preserved morphology [10]. In view of the latter it is possible to make an assumption that the revolved hypoinsulinemia in experimental animals after ten months' light exposure is a result of further destructive process in the endocrine part of the pancreas.

Thus, the course of MS in experimental animals against the background of long-term hypopinealism is associated with the further progression of glucose homeostasis disorders. The determined hyperglycemia against the background of hypoinsulinemia over the day in the experimental animals under the condition of ACL over ten months points to deeper changes in the glucose metabolism in the experimental rabbits compared with ones that have been identified as a result of five months of ACL.

It is known that MS is considered as a premorbid state of type 2 diabetes. According to contemporary views about the pathogenesis of insulin independent diabetes, the manifestation of this disease in humans is associated with a significant decrease in the number of pancreatic β -cells against the background of spontaneous evolution from relative to absolute insulin deficiency with persistent insulin resistance [13]. Taking into account the latter, the above results of changes in functional activity of pancreatic β -cells and glycemia in course of MS against a background of long-term hypopinealism along with the earlier established morphological disturbances in Langerhans' islets structure during the formation of MS under these conditions [10], it is possible to conclude that to some extent the carbohydrate metabolism changes in the experimental animals are similar to those ones belonging to pathogenesis of type 2 diabetes mellitus in humans.

The results of this work coincide with the scientific literature data on receptor-mediated melatonin modulation of daily rhythm of physiological functioning pancreatic β -cells and, accordingly, the participation of this hormone in the regulation of carbohydrate metabolism [1, 11, 12]. Moreover, a determined increase in the expression of membrane melatonin receptors in islets of Langerhans among the patients with type 2 diabetes and a positive therapeutic effect on carbohydrate metabolism in Zucker diabetic fatty rats additionally indicate that the prolonged

violation of this hormone product by the pineal gland can have profound negative consequences for the state of glucose homeostasis [14].

Conclusions

1. Sustained melatonin deficiency under the condition of the metabolic syndrome leads to the insulinemia daily rhythm levelling provided by hypoinsulinemia over the day.

2. The course of metabolic syndrome against the background of long-term hypopinealism induced by round-the-clock lighting for ten months is associated with the glycemia daily rhythm levelling due to hyperglycemia during both the day and night times.

3. Hyperglycemia and hypoinsulinemia as a result of the metabolic syndrome course in long-term hypopinealism should be considered as a prerequisite for manifestation of type 2 diabetes mellitus hereafter.

Prospects for further research

Research will continue in the chosen scientific direction.

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Information about authors:

Bondarenko L. - Doctor of biological sciences, senior researcher fellow, SI "V. Danilevsky Institute for Endocrine Pathology Problems National Academy of Medical Sciences of Ukraine", Kharkiv

Mishchenko T., PhD, assistant of the department of socio-humanitarian and biomedical disciplines of the Kharkiv Institute of Medicine and Biomedical Sciences of PHEE "Kyiv Medical University".

Відомості про авторів:

Бондаренко Л. - д. біол. н., старший науковий співробітник, Інститут проблем ендокринної патології ім. В. Данилевського Національної академії медичних наук України", м. Харків

Мищенко Т., к. біол. н., асистент кафедри соціально-гуманітарних і біомедичних дисциплін Харківського інституту медицини та біомедичних наук ПВНЗ "Київський медичний університет".

Сведения об авторах:

Бондаренко Л. - д. биол. н., старший научный сотрудник, Институт проблем эндокринной патологии им. В. Данилевского Национальной академии медицинских наук Украины", г. Харьков

Мищенко Т., к. биол. н., ассистент кафедры социально-гуманитарных и биомедицинских дисциплин Харьковского института медицины и биомедицинских наук ЧВУЗ "Киевский медицинский университет".

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Рецензент – проф. Н.В. Пашковська

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