

HYPOPINEALISM AND ARTERIAL HYPERTENSION: CHRONOBIOLOGICAL AND AGE ASPECTS*

T. Mishchenko^{1,2}, L. Bondarenko²

¹ V. N. Karazin Kharkiv National University, Kharkiv, Ukraine;

² SI «V. Danilevsky Institute for Endocrine Pathology Problems of the NAMS of Ukraine»,
Kharkiv, Ukraine
t.mishchenko@kmu.edu.ua

It is known that age hypopinealism, i.e. decrease in the pineal gland functioning characterised first of all by progressive melatonin deficiency, develops in mammals at the downward ontogeny stage [1, 2]. Since physiological fluctuations of the pineal gland hormone synthesis and secretion level is one of the conditions for the coherence of the daily rhythms of the organs/systems work [2, 3], it is considered that pineal gland hypofunction plays an important role in the pathogenesis of age-associated diseases, in particular arterial hypertension (AH) [2]. It is known that apparently healthy elderly people have higher pineal gland melatonin-forming function than the ones with AH [4]. At the same time AH is not only an old people's disease, it is also disease of mature aged persons; thus the phrase «AH has looked younger» is established.

In our previous researches done on mature rabbits with hypopinealism induced by long-lasting (for 4–5 months) 24-hour lighting the development of AH was established in case of single blood pressure (BP) measuring in the day-time. In the further chronobiological studies we have found that the long-lasting disruption of light mode leads to desynchronosis in animals and against this AH develops according to the type of «non-dipper» (i.e. leveling of BP circadian rhythm) [5]. This type of AH is specific to elderly humans with melatonin deficient typical for age hypopinealism [1].

Therefore, the purpose of this study was to take a comparative assessment of changes in circadian rhythms of blood pressure in rabbits of different ages (mature and old) with induced long-lasting 24-hour lighting or age hypopinealism.

* The work is done in accordance with the planned scientific theme of chronoendocrinology laboratory of State Institution «V. Danilevsky Institute for Endocrine Pathology Problems of the NAMS of Ukraine» «To research mechanisms of senilism in hypopinealism: chronobiological aspects» (state registration number 0113U001282).

The institution financing the work is NAMS of Ukraine.

The authors guarantee corporative responsibility for the published article.

The authors guarantee absence of competing interests and their own financial interest while doing the research and writing the article.

The manuscript was received by the editorial staff 2.07.2019.

MATERIALS AND METHODS

The research is done on 30 male rabbits aged 4-5 months at the beginning of the experiment. The animals form three groups: Group I — mature, Group II — old, Group III — mature with hypopinealism. The rabbits were kept in standard vivarium conditions except the lighting mode. Animals of groups I and II were kept upon natural day-night cycle for 10 months and 3 years and a half respectively. In rabbits of group III hypopinealism was modeled by keeping the animals in conditions of 24-hour lighting (during the daylight it was natural sunlight, in the darkness it was electric light making the illumination of 30–40 lux) for 10 months [6].

The study of the BP daily rhythm in rabbits is based on systolic blood pressure (SBP) measurement on the central ear artery according to R.T. Grant and P. Rothschild's method in our modification [7] at four time points: morning (7 a.m.–8 a.m.), day (1 p.m.–2 p.m.), evening (7 p.m.–8 p.m.), night (1 a.m.–2 a.m.). The BP circadian rhythm analysis was done by using the following characteristics:

- 1) acrophase (maximal SBP);
- 2) batiphase (minimal SBP);
- 3) mesor (average daily blood pressure);
- 4) amplitude (maximal SBP displacement relatively to the mesor);
- 5) the degree of blood pressure reducing (DBPR).

$$DBPR = \frac{BP_{\text{day}} - BP_{\text{night}}}{BP_{\text{day}}} \times 100 \%$$

RESULTS AND THEIR DISCUSSION

The features of SBP change within 24 hours in the mature and old rabbits as well as in the mature ones with AH induced by hypopinealism, are given in Table 1.

As Table 1 shows, the mature rabbits have a clearly marked SBP circadian rhythm caused by the physiological BP decrease in the darkness versus the morning figure.

The old animals have the BP level increase at the different day periods, but the most expressed in the evening and at night (by 33.8 and 35.3 % compared to the corresponding quantities in the mature rabbits) that provides levelling of the BP rhythm and gives

An incandescent lamp with the power of 100 W was used as an artificial light source. The illumination in animals' cages was determined with luxmeter «Yu-117». BP was evaluated in the natural light during the day-time and in the red light over the night-time due to the fact that red light has minimal effect on melatonin production in the pineal gland compared with the other colors of the visible-light spectrum [8].

All procedures with the animals were done according to the European Convention for Protection of Vertebrate Animals used for experimental and other scientific purposes.

Statistical analysis of the digital material was done with parametric and non-parametric methods. The verification of the data distribution according to the Gauss law was carried out using the Shapiro-Wilk test.

The comparison of groups with the data normal distribution was done using unpaired Student's t-test. The Mann-Whitney U-test was used to analyze the data which distribution did not correspond to the Gauss law. The one-way analysis of variance was used for multiple comparisons. The results are given in the form of $(\bar{X} \pm S_{\bar{X}})$. The level of significance (p) was expected as $p \leq 0.05$ for determining the difference between groups that was statistically significant.

the reasons for classifying the AH type as «non-dipper».

As shown in Table 1, the rabbits of the third group have the same SBP changes as those ones obtained in the old animals.

In other words, development of AH type «non-dipper» was revealed in the old animals with age hypopinealism and in the mature rabbits with hypopinealism induced by 24-hour lighting.

The detailed characterization of the BP circadian variations based on the most important parameters used in chronobiology is presented in Table 2.

Table 1

**Circadian Rhythm of Systolic Blood Pressure
in Rabbits During Different Physiological Development Periods
and in Rabbits with Hypertension Induced by Hypopinealism (mm Hg), ($\bar{X} \pm S_{\bar{x}}$), n = 10**

Group of Animals	Day Time			
	morning	day	evening	night
I. Mature	50.6 ± 0.9	52.4 ± 0.7	46.4 ± 1.4*	45.0 ± 1.1*
II. Old	62.0 ± 1.4 pI-II < 0.001	61.0 ± 1.0 pI-II < 0.001	61.0 ± 0.8 pI-II < 0.001	60.0 ± 0.9 pI-II < 0.001
III. Mature with hypopinealism	60.7 ± 1.6 pI-III < 0.001 pII-III > 0.05	61.1 ± 0.5 pI-II < 0.001 pII-III > 0.05	62.1 ± 0.6 pI-II < 0.001 pII-III > 0.05	60.9 ± 0.2 pI-II < 0.001 pII-III > 0.05

Note:

* it is p < 0.05 compared to the morning figure.

Table 2

**The Main Parameters of Circadian Rhythm of Systolic Blood Pressure
in Rabbits During Different Periods of Ontogenesis and in Rabbits
with Arterial Hypertension Against Hypopinealism, ($\bar{X} \pm S_{\bar{x}}$), n = 10**

Group of animals	Acrophase, mm Hg	Batiphase, mm Hg	Amplitude, mm Hg	SBP drop degree, %	Mesor, mm Hg
I. Mature	52.4 ± 0.7 (day)	45.0 ± 1.1 (night)	8.2 ± 0.3	14.2 ± 0.7	48.7 ± 0.9
II. Old	62.0 ± 1.4 (morning) pI-II < 0.001	60.0 ± 0.9 (night) pI-II < 0.001	2.5 ± 0.2 pI-II < 0.001	1.6 ± 0.3 pI-II < 0.001	61.2 ± 0.4 pI-II < 0.001
III. Mature with hypopinealism	62.1 ± 0.6 (evening) pI-III < 0.001 pII-III > 0.05	60.7 ± 1.6 (morning) pI-III < 0.001 pII-III > 0.05	1.8 ± 0.2 pI-III < 0.001 pII-III > 0.05	0.7 ± 0.5 pI-III < 0.001 pII-III > 0.05	61.3 ± 0.4 pI-III < 0.001 pII-III > 0.05

As shown in Table 2, the SBP circadian rhythm in the mature rabbits has the biphasic due to achievement of BP acrophase in the afternoon and batiphase at night. The absolute indexes of the amplitude, DBPR and mesor as well also point at the sinusoidal nature of the SBP fluctuations in the mature rabbits within 24 hours.

The results of this study fragment coincide with the scientific reports about the occurrence of the SBP circadian rhythm in the mature rabbits as well as in healthy people [9, 10]. It is well-known that DBPR (about 10–20 %) at night is a physiological feature of human body [10]. Thus, it is accepted to classify this kind of BP circadian rhythm as «dipper» type using the results of daily BP monitoring as a diagnostic criterion in a clinical practice [10]. Hence, the results of the study fragment point at the fact that SBP circadian variations in the mature

rabbits are similar to the BP daily rhythm of «dipper» type in humans in the appropriate age period.

Reaching old age the age-related changes occurring in rabbits' body are also reflected in the change of a BP level within 24 hours. In those rabbits the SBP mesor increase has been found versus the value in the mature rabbits as well as the acrophase displacement from the day-time to the morning (Table 2).

Moreover as a result of the SBP circadian rhythm change in the old animals the decrease of amplitude by 3.3 times was revolved compared to corresponding quantity in the mature rabbits. The latter induces the DBPR falling at night in those animals. The specified characteristics of a SBP circadian rhythm in the rabbits at the descending ontogenesis stage refer to SBP daily rhythm leveling revealed in the mature rabbits.

The obtained data correspond to the results of the clinical research study that show the development of AH «non-dipper» type described by the insufficient DBPR at night ($< 10\%$) or its deficiency in healthy people at ageing [10]. The results of clinical experimental study show existence of the general biological mechanism of AH pathogenesis based on the decrease of melaton-forming function of a pineal gland [1]. The obtained data about the acrophase displacement to the morning in the old animals are representative ones taking into account the fact that age is classified as an independent risk factor of BP rapid increase in the early morning time in human that, in turns, is associated with onset of cardiovascular catastrophes at the particular day time at the descending ontogenesis stage with untreated AH [11].

The changes of a SBP circadian rhythm in the old animals are similar to those ones that belong to humans in the senility and it is classified as SBP circadian rhythm «non-dipper» type.

While comparing the studied characteristics of a SBP circadian rhythm in the mature rabbits with AH induced by long-lasting hypopinealism and in the old animals the difference between them was not revealed (see table 2). But it is to be noted that the acrophase was at the evening and batiphase was at the morning in rabbits with AH against hypopinealism when corresponding characteristics were obtained in the afternoon and at night in the mature rabbits. At the same time the displacement only maximum of SBP to the morning time was revealed in the old animals and probably that is a risk of cardiovascular disease development in those animals. The feature of the acrophase displacement to the evening in the animals with hypopinealism probably can also be interpreted as manifestation of risk of cardiovascular complication development but upon a total suppression of the pineal gland melatonin-forming function and, consequently,

«loss» of the day time concept for a body. It is well-known that an important property of the circadian system is the development in body of a «biological day» and a «biological night» cyclically alternating with their transitions characterized by relatively sharp hormonal, electrophysiological, behavioral changes [3]. The revealed acrophase displacement to the evening in the rabbits with AH against hypopinealism have an extra importance taking into account the results of our previous studies that gave the evidence of chronic heart failure development, endothelial disfunction and atherosclerosis in those animals [12].

Thus the obtained results assume that AH development in rabbits against untreated hypopinealism induced by a long-term 24-hour lighting is connected with the leveling of SBP circadian rhythm. Hence a long-term 24-hour lighting should be considered as one of their senilism risks.

In conclusion, the pathophysiological mechanisms of the insufficient DBPR at night time are not completely established currently. But it is known that pathogenesis of AH associated with melatonin deficiency and the BP circadian rhythm «non-dipper» type is related with the disruption of nervous system functioning and an excessive catecholamines production as well as the development of insulin resistance, activation of the renin-angiotensin system [13]. Taking into account the results of chronobiological studies about the changes of adrenaline and noradrenaline concentrations as well as metabolic syndrome formation in mature rabbits with hypopinealism, it is possible to confirm that the AH development mechanism against this background is connected with a long-term and excessive activation of sympathoadrenal system in the same manner as it occurs in ageing and stress (stress-age syndrome according to V. Frolkis) and insulin resistance development [14, 15].

CONCLUSIONS

1. Hypopinealism regardless of the reasons its formation (age-dependent or induced by a long-lasting 24-hour lighting) causes the development of arterial hypertension of «non-dipper» type in rabbits.
2. The occurrence of arterial hypertension associated with hypopinealism induced with a long-lasting light exposure during the darkness in mature rabbits should be considered as one of their premature ageing symptoms.

REFERENCES

1. Korkushko OV, Bondarenko LO, Shatylo VB, et al. *Zhurn AMN Ukraïny* 2008; 14(2): 373-381.
2. Anisimov VN. *Uspehi Fiziologicheskikh Nauk* 2008; 39(4): 52-76.
3. Tsang AH, Barclay J, Oster H. *J Mol Endocrinol* 2014; 52(1): 16-32.
4. Shatilo VB, Antonjuk-Shheglova IA, Bondarenko EV, et al. *Krovoobig ta Gemostaz* 2007; 4: 18-25.
5. Bondarenko LA, Mishhenko TV. *Patologicheskaja Fiziologija i Jeksperimental'naja Terapija* 2015; 1: 46-49.
6. Gubina-Vakulik GI, Bondarenko LA, Sotnik NN. *Uspehi Gerontologii* 2007; 1: 92-95.
7. *Patent 83426 UA*. Pristriy dlja vimirjuvannja arteri- al'nogo tisku u kroliv.
8. Brainard GC, Richardson BA, King TS, et al. *Brain Res* 1984; 5: 333-339.
9. Van den Buuse M, Malpas SC. *Physiol Behav* 1997; № 62(1). P. 83-89.
10. Viera AJ, Zhu S, Hinderliter AL, et al. *J Am Soc Hy- pertens* 2011; 5(1): 48-55. doi: 10.1016/j.jash.2010.12.002.
11. Lee DH, Ihm SH, Youn HJ. *Korean Circ J* 2009; 39(8): 322-327. doi: 10.4070/kcj.2009.39.8.322.
12. Bondarenko LO, Kondakov IK, Gladkova AI, et al. *Bu- kov Med Visn* 2009; 13(4): 41-45.
13. Simko F, Reiter RJ, Pechanova O. et al. *Front Biosci* 2013; 18: 616-625.
14. Bondarenko LA, Gorbach TV, Kozak VA. *Uspehi Ge- rentologii* 2014; 27(4): 625-630.
15. Mishhenko TV, Gladkyh OI, Poltorak VV, Bondaren- ko LO. *Endokrynologija* 2015; 2: 494-500.

**HYPOPINEALISM AND ARTERIAL HYPERTENSION:
CHRONOBIOLOGICAL AND AGE ASPECTS**

T. Mishchenko^{1,2}, L. Bondarenko²

¹ V. N. Karazin Kharkiv National University, Kharkiv, Ukraine;

² State Institution «V. Danilevsky Institute for Endocrine Pathology Problems of the NAMS of Ukraine»,
Kharkiv, Ukraine
t.mishchenko@kmu.edu.ua

The research is done on the mature male rabbits divided into three groups: mature animals (group I), old animals (group II), and mature animals with hypopinealism (group III). The rabbits in the I and II group were kept in the natural light conditions (the former ones for a period of 10 months and the latter ones for 3 years and a half). Hypopinealism was induced in rabbits (group III) by 24-hour lighting for 10 months. Blood pressure was measured on the animals' central ear artery four times a day (in the morning [from 07:00 a.m. until 08:00 a.m.], in the afternoon [from 01:00 p.m. until 02:00 p.m.], in the evening [from 07:00 p.m. until 08:00 p.m.] and at night [from 01:00 a.m. until 02:00 a.m.]) every month. The blood pressure circadian rhythm was analyzed on the basis of the quantities of acrophase, batiphase, mesor, amplitude, degree of blood pressure reducing. It was revealed that the blood pressure indices in the rabbits with hypopinealism and in the old animals were similar during the day, but the quantities exceeded corresponding values in the mature animals. Moreover, the indices of blood pressure circadian rhythm in the rabbits with hypopinealism and in the old animals show leveling of the biphasic circadian pattern of the parameter identified in the mature rabbits. Thereby, hypopinealism (induced by age or long-lasting 24-hour lighting) causes the development of arterial hypertension of «non-dipper» type in the rabbits. Consequently, formation of hypertension against hypopinealism induced by the long-lasting low-intensity light exposure during the darkness is one of the animal senilism symptoms.

Key words: hypopinealism, arterial hypertension, circadian rhythm, blood pressure.

ГИПОПИНЕАЛИЗМ ТА АРТЕРІАЛЬНА ГІПЕРТЕНЗІЯ: ХРОНОБІОЛОГІЧНІ ТА ВІКОВІ АСПЕКТИ

Мищенко Т.^{1,2}, Бондаренко Л.²

¹ Харківський національний університет ім. В.Н. Каразіна, м. Харків, Україна;

² ДУ «Інститут проблем ендокринної патології ім. В.Я. Данилевського НАМН України»,
м. Харків, Україна
t.mishchenko@knu.edu.ua

Дослідження виконано на самцях кроля, які були розподілені на три групи: I — статевозрілі, II — старі, III — статевозрілі з гіпопінеалізмом. Тварин I і II груп утримували за умови природного світлового режиму впродовж 10 місяців та 3,5 років, відповідно. У кролів III групи моделювали гіпопінеалізм шляхом цілодобового освітлення протягом 10 місяців. У тварин усіх груп щомісяця чотири рази на добу: вранці (07:00 год — 08:00 год), вдень (13:00 год — 14:00 год), ввечері (19:00 год — 20:00 год) та вночі (01:00 год — 02:00 год), вимірювали кров'яний тиск на центральній артерії вуха. Аналіз добового ритму артеріального тиску був проведений на основі показників акрофази, батіфази, мезора, амплітуди, а також ступеня зниження артеріального тиску. Встановлено, що показники артеріального тиску у кролів з гіпопінеалізмом та у старих тварин не мають відмінностей протягом доби, проте перевищують відповідні величини, зареєстровані у статевозрілих тварин. В результаті проведення аналізу параметрів добового ритму кров'яного тиску у кролів з гіпопінеалізмом та у старих тварин було отримано докази нівелювання двофазної циркадної ритмічності артеріального тиску, виявленої у статевозрілих тварин. Таким чином, гіпопінеалізм (віковий або індукований тривалим цілодобовим освітленням) викликає у кролів розвиток гіпертензії за типом «non-dipper». Відповідно, формування артеріальної гіпертензії на фоні гіпопінеалізму, викликаного тривалою низькоінтенсивною світловою експозицією в темну пору доби, є однією з ознак передчасного старіння тварин.

Ключові слова: гіпопінеалізм, артеріальна гіпертензія, циркадний ритм, артеріальний тиск.

ГИПОПИНЕАЛИЗМ И АРТЕРИАЛЬНАЯ ГИПЕРТЕНЗИЯ: ХРОНОБИОЛОГИЧЕСКИЕ И ВОЗРАСТНЫЕ АСПЕКТЫ

Мищенко Т.^{1,2}, Бондаренко Л.²

¹ Харьковский национальный университет им. В.Н. Каразина, г. Харьков, Украина;

² ГУ «Институт проблем эндокринной патологии им. В.Я. Данилевского НАМН Украины»,
г. Харьков, Украина
t.mishchenko@knu.edu.ua

Исследование выполнено на кроликах-самцах, разделенных на три группы животных: I — половозрелые, II — старые, III — половозрелые с гипопинеализмом. Животных I и II групп содержали в условиях естественного светового режима в течение 10 месяцев и 3,5 лет, соответственно. У кроликов III группы моделировали гипопинеализм путем круглосуточного освещения в течение 10 месяцев. У животных всех групп ежемесячно четыре раза в сутки: утром (07:00 ч — 08:00 ч), днем (13:00 ч — 14:00 ч), вечером (19:00 ч — 20:00 ч) и ночью (01:00 ч — 02:00 ч), измеряли кровяное давление на центральной артерии уха. Анализ суточного ритма артериального давления был проведен на основании показателей акрофазы, батифазы, мезора, амплитуды, а также степени снижения артериального давления. Установлено, что показатели артериального давления у кроликов с гипопинеализмом и у старых животных не имеют различий в течение суток, однако превышают соответствующие величины, зарегистрированные у половозрелых животных. В ходе анализа параметров суточного ритма кровяного давления у кроликов с гипопинеализмом и у старых животных были получены доказательства нивелирования двухфазной циркадной ритмичности артериального давления, выявленной у половозрелых животных. Таким образом, гипопинеализм (возрастной либо индуцированный длительным круглосуточным освещением) вызывает у кроликов развитие гипертензии по типу «non-dipper». Следовательно, формирование артериальной гипертензии на фоне гипопинеализма, вызванного длительной низкоинтенсивной световой экспозицией в темное время суток, является одним из признаков преждевременного старения животных.

Ключевые слова: гипопинеализм, артериальная гипертензия, циркадный ритм, артериальное давление.