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Sex differences in the cardiac cholinergic response to adrenalin-induced myocardial necrosis and light desynchronosis

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

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The aim was to identify sex differences in the cardiac response to cholinergic stimuli in the development of myocardial necrosis and light desynchronosis.

Materials and methods. Experiments were conducted in 144 albino rats (72 males, 72 females). Myocardial necrosis was induced by adrenaline (0.5 mg/kg, intramuscularly).

The intensity of bradycardia that occurred as a response to intravenous acetylcholine injection and electrical stimulation of vagus nerve was measured. The animals were divided into 4 groups: 1 – rats that were in a light balance (day/night); 2 – rats exposed to light deprivation for 10 days; 3 – rats exposed to permanent lighting for 10 days; 4 – animals exposed to permanent lighting for 10 days and injected with melatonin (5 mg/kg) intraperitoneally 1 hour before the necrosis modeling.

Results. The development of myocardial necrosis when occurring in light deprivation was characterized by an increased responsiveness of the rat heart to cholinergic stimulation due to an increase in both cholinoreceptors sensitivity and release of acetylcholine from the vagus nerve terminals in females, and due to only an increased cholinoreceptors sensitivity in males. The development of myocardial necrosis in rats exposed to permanent lighting was characterized by significantly higher sensitivity of cholinergic structures of the heart than that in light balance, especially in females. Melatonin injection in the development of myocardial necrosis contributed to the heart cholinergic structures response to the stimulation, which was close to that observed in conditions of light balance, though it remained somewhat higher: due to the facilitation of acetylcholine release from vagus nerve in females, and it was combined with a higher sensitivity of cholinoreceptors in males.

Conclusions. The development of myocardial necrosis in light desynchronosis (light deprivation or permanent lighting) causes an increased heart response to cholinergic stimulation in rats. Such effects are stronger, especially in females in conditions of permanent lighting. The injection of melatonin in rats with myocardial necrosis and permanent lighting results in an approximation (but not restoration) of the heart sensitivity to cholinergic stimuli parameters to those that are observed in light balance. However, the heart response to cholinergic stimulation remains higher, especially in males.

Ключові слова: некроз міокарда, світловий десинхроноз, чутливість холінорецепторів, n. vagus, стать.

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Статеві відмінності холінергічних кардіальних реакцій при розвитку адреналін-індукованого некрозу міокарда на тлі світлового десинхронозу

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Мета роботи – встановити статеві відмінності реагування серця на холінергічні стимули під час розвитку некрозу міокарда на тлі світлового десинхронозу.

Матеріали та методи. Досліди виконали на 144 щурах-альбіносах (72 самці, 72 самиці). Некроз міокарда викликали введенням адреналіну (0,5 мг/кг внутрішньом'язово). Визначали інтенсивність брадикардії, що виникала під час внутрішньовенного введення ацетилхоліну та електричної стимуляції n. vagus. Тварин поділили на 4 групи: 1 – щури, які перебували в умовах світлового балансу (день/ніч); 2 – щури, які 10 днів перебували в умовах світлової депривації; 3 – щури, які 10 днів перебували в умовах перманентного освітлення; 4 – тварини, які 10 днів перебували в умовах перманентного освітлення, але за 1 годину до моделювання некрозу отримували мелатонін (5 мг/кг внутрішньочеревно).

Результати. Під час розвитку некрозу міокарда на тлі світлової депривації реакція серця щурів на холінергічні впливи збільшувалася: в самиць – завдяки збільшенню чутливості холінорецепторів і вивільнення більшої кількості ацетилхоліну з закінчень n. vagus, а в самців – тільки внаслідок збільшення чутливості холінорецепторів. Розвиток некрозу міокарда в умовах перманентного освітлення характеризувався суттєвішою, ніж за світлового балансу, чутливістю холінергічних структур серця, особливо в самиць. Застосування мелатоніну сприяло тому, що при розвитку некрозу міокарда реакція холінергічних структур серця на стимуляцію наближалася до зареєстрованої в умовах світлового балансу, хоча й залишалася дещо більшою: у самиць – унаслідок полегшеного вивільнення ацетилхоліну з n. vagus, а в самців поєднувалася з більшою чутливістю холінорецепторів.

Висновки. Розвиток некрозу міокарда на тлі світлового десинхронозу (світлова депривація чи перманентне освітлення) викликає посилення реагування серця щурів на холінергічну стимуляцію. Суттєвішими такі ефекти є в умовах перманентного освітлення, особливо в самиць. Застосування мелатоніну під час моделювання некрозу міокарда на тлі перманентного освітлення забезпечує наближення (але не відновлення) параметрів, що характеризують чутливість серця до холінергічних стимулів, до зареєстрованих в умовах світлового балансу. Реакція серця на холінергічну стимуляцію залишається дещо більшою, особливо в самців.

Половые отличия холинергических кардиальных реакций при развитии адреналин-индуцированного некроза миокарда на фоне светового десинхроноза

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Ключевые слова: некроз миокарда, световой десинхроноз, чувствительность холинорецепторов, n. vagus, пол.

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Цель работы – установить половые отличия реагирования сердца на холинергические стимулы при развитии некроза миокарда на фоне светового десинхроноза.

Материалы и методы. Опыты провели на 144 крысах-альбиносах (72 самца, 72 самки). Некроз миокарда вызывали введением адреналина (0,5 мг/кг внутримышечно). Определяли интенсивность брадикардии при внутривенном введении ацетилхолина и электрической стимуляции n. vagus. Животных поделили на 4 группы: 1 – крысы, которые находились в условиях светового баланса (день/ночь); 2 – крысы, которые 10 дней находились в условиях световой депривации; 3 – крысы, которые 10 дней находились в условиях перманентного освещения; 4 – крысы, которые 10 дней находились в условиях перманентного освещения, но за 1 час до моделирования некроза получали мелатонин (5 мг/кг внутрибрюшинно).

Результаты. При развитии некроза миокарда на фоне световой депривации реакция сердца крыс на холинергическую стимуляцию увеличивалась: у самок – за счет увеличения чувствительности холинорецепторов и освобождения большего количества ацетилхолина из n. vagus, а у самцов – только за счет увеличения чувствительности холинорецепторов. Развитие некроза миокарда в условиях перманентного освещения характеризовалось более существенной, чем при световом балансе, чувствительностью холинергических структур сердца к функциональным влияниям, особенно у самок.

Использование мелатонина способствовало тому, что при развитии некроза миокарда реакция холинергических структур сердца приближалась к показателям, зарегистрированным при световом балансе, хотя они оставались большими: у самок – за счет освобождения большего количества ацетилхолина из окончаний n. vagus, а у самцов сочеталась с большей чувствительностью холинорецепторов.

Выводы. Развитие некроза миокарда на фоне светового десинхроноза (световая депривация или перманентное освещение) вызывает усиление реакции сердца крыс на холинергическую стимуляцию. Более существенными такие эффекты зарегистрированы в условиях перманентного освещения, особенно у самок. Использование мелатонина при моделировании некроза миокарда на фоне перманентного освещения способствует приближению (но не восстановлению) параметров, которые отображают чувствительность сердца к холинергической стимуляции в условиях светового баланса. Реакция сердца на холинергическую стимуляцию остается более существенной, особенно у самцов.

The prevalence of cardiovascular diseases in the list of high mortality causes is the main characteristic nowadays [1–3]. Stress takes the lead among the risk factors for coronary heart disease and myocardial infarction. Pineal gland dysfunction and abnormal circadian rhythm are worthy of being noted among the causes of stress [4]. Light desynchronosis occurs in shiftwork, jetlag and prolonged computer work. All above mentioned causes a significant exertion of stress adaptation mechanisms, their rapid exertion and cardiovascular system dysfunction in impaired light regime [5,6].

Effective body adaptation to stress is possible due to the pineal gland hormone melatonin which is synthesized mainly during nighttime sleep. There is a good deal of evidence that melatonin reduces the effects of stress. It has been shown that melatonin desensitizes adrenergic receptors, reduces free radical oxidation due to antioxidant properties and ability to stabilize mitochondrial membranes, and enhances the realization of cholinergic effects at the central and peripheral levels, providing cardioprotection against stress, hypercatecholaminemia, ischemia/hypoxia [7–11].

It is also known that the incidence of cardiovascular pathology in light desynchronosis depends on the sex and occurs more frequently in men [12]. All these facts indicate the relevance of research studies aimed at analyzing the effect of light balance disorders on cardiovascular diseases development and sex-specific aspects of this issue. This will foster the development of not only cardiology, but also gender medicine.

Aim

The aim was to identify sex differences of the cardiac response to cholinergic stimuli in the development of myocardial necrosis and light desynchronosis.

Materials and methods

Experiments were conducted in 144 albino rats (72 males, 72 females) weighing 220–290 g, which were on a standard ration in vivarium conditions. Animals were divided into 4 groups: 1 – rats that were in a light balance (day/night); 2 – rats exposed to light deprivation for 10 days; 3 – rats exposed to permanent lighting for 10 days; 4 – animals exposed to permanent lighting for 10 days and injected with melatonin (a single dose of 5 mg/kg) intraperitoneally 1 hour before the myocardial necrosis (MN) modeling [7].

Light balance model: the animals were housed on day/night cycles for 10 days; cycle “Day” consisted of a 12-hour (from 7.00 to 19.00) 500 lux light, cycle “Night” lasted 12 hours (from 19.00 to 7.00) at 0.5–1.0 lux light. Model of light deprivation: animals were left in 0.5–1.0 lux light for 10 days. Permanent lighting model: animals were exposed to 500 lux light for 10 days. On the 11th day, adrenaline-induced myocardial necrosis was modeled by adrenaline intramuscular injection (0.5 mg/kg).

The basic studies were performed 1 and 24 hours after injection, including standard lead II ECG registration using the Cardiolab-SE computer complex (Kharkiv,

Table 1. Indicators of the rat heart response to cholinergic stimulation in the development of myocardial necrosis in light balance, M ± m

Indices	Sex	Control, n = 6	MN 1 hour, n = 6	MN 24 hours, n = 6
BI_{vn}	♂	6.85 ± 0.31[2]	6.89 ± 0.23[2]	7.49 ± 0.35
	♀	8.25 ± 0.45[2]	8.90 ± 0.36[2]	6.68 ± 0.40[1]
BI_{ACh}	♂	3.94 ± 0.35	3.88 ± 0.30	3.96 ± 0.26
	♀	3.94 ± 0.42	3.52 ± 0.38	4.64± 0.38
BD_{ACh} (sec)	♂	18.8 ± 1.0[2]	16.4 ± 1.3	17.9 ± 1.2
	♀	25.7 ± 0.9[2]	16.3 ± 0.7[1]	17.8 ± 0.8[1]

[1]: significance of the differences in comparison with the control, P < 0.05; [2]: significance of the differences between sexes, P < 0.05.

Table 2. Indicators of the rat heart response to cholinergic stimulation during the development of myocardial necrosis in light deprivation, M ± m

Indices	Sex	Control, n = 6	MN 1 hour, n = 6	MN 24 hours, n = 6
BI_{vn}	♂	6.87 ± 0.21[3]	6,08 ± 0.29[3]	6.94 ± 0.24[3]
	♀	12.03 ± 0.30[2,3]	10.49 ± 0.37[1,2,3]	8.20 ± 0.22[1,2,3]
BI_{ACh}	♂	8.21 ± 0.33[2]	5.82 ± 0.27[1,2]	6.01 ± 0.35[1,2]
	♀	7.88 ± 0.38[2]	6.19 ± 0.21[1,2]	5.16 ± 0.25[1]
BD_{ACh} (sec)	♂	12.9 ± 0.7[2,3]	14.4 ± 1.3[3]	13.5 ± 1.2[3]
	♀	24.6 ± 1.7[3]	20.7 ± 1.3[2,3]	20.2 ± 1.1[2,3]

[1]: significance of the differences in comparison with the control, P < 0.05; [2]: significance of the differences in comparison with group 1, P < 0.05; [3]: significance of the differences between sexes, P < 0.05.

Ukraine). The cardiac response to exogenous acetylcholine (ACh) injected into the jugular vein (50 mg/kg to a maximum of 0.1 ml/100 g of animal body weight) was evaluated by bradycardia intensity (BI_{ACh}) and bradycardia duration (BD_{ACh}). BI_{ACh} was calculated by the formula:

$$BI_{ACh} = (R–R)max / (R–R)m \quad (1)$$

The value of *(R–R)m* was equal to the arithmetic mean of the (R-R) intervals recorded on the ECG before the ACh injection; the value of *(R–R)max* was equal to the maximum (R–R) value after the ACh injection.

This indicator reflects the sensitivity of cholinoreceptors in the sinus node. BD_{ACh} is the period between the onset and termination of a negative chronotropic effect (expressed in seconds), reflecting the duration of functional desensitization of cholinoreceptors. Heart response to electrical stimulation of the right peripheral segment of vagus nerve was estimated by bradycardia intensity (BI_{vn}).

Electrical stimulation was performed using an ELS-2 (electrical laboratory stimulator). Operation parameters of the device: electrical impulse duration – 1 ms, impulse delay – 1 ms, impulse amplitude – 2 V, impulse frequency – 50 Hz. The peripheral segment of the nerve was isolated at the level of the lower cartilage of the larynx and placed on copper electrodes (a pole separation was 4 mm). The nerve was stimulated for 60 seconds. BI_{vn} was calculated by the formula (1), but the value of *(R-R)m* was equal to the arithmetic mean of the (R–R) intervals recorded on the ECG before electrical stimulation of vagus nerve; *(R–R) max* – maximum value (R–R) during vagus nerve stimulation.

This parameter reflects ACh content in the presynaptic vagus nerve terminals. Experiments studying the heart response to cholinergic stimulation were carried out in anesthetized animals (thiopental sodium, 40 mg/kg, intraperitoneally).

The experiments on heart response to electrical stimulation were carried out in anesthetized animals (thiopental sodium 40 mg/kg of animal body weight, intraperitoneally) and followed the National "General Ethical Principles of Animal Experiments" (Ukraine, 2001) in accordance with the 2010/63EU Directive of the European Parliament and of the Council of September 22, 2010 on the protection of animals used for scientific purposes [13]. The experiments were conducted on the basis of the Central Research Laboratory (CRL) of the Ternopil National Medical University. All devices used for study were certified and had undergone annual metrological control (certificate of technical competence No. 001/18 dated September 26, 2018, valid until September 26, 2023).

Statistical processing was performed using a table processor Microsoft Excel 2016 (Microsoft Corp., USA). For all the parameters, the arithmetic mean (M), its dispersion and mean error (m) were calculated. To determine the significance of differences between the study results in the experimental groups of animals, the Student's coefficient (t) was calculated, after that, the significance of the differences between the samples (p) and the confidence interval of the mean according to the Student's t-distribution tables were calculated. P_{St} values < 0.05 were considered statistically significant (program BioStat, AnalystSoft Inc., version 6) [14].

Results

Analysis of the animal data obtained in group 1 showed that male BI_{vn} did not change in MN development and light balance (*Table 1*).

Female BI_{vn} decreased by 24 % within 24 h of MN. Neither male nor female BI_{ACh} changed in the development of MN. BD_{ACh} was changed only in females, it represented a 58 % decrease (within 1 h of MN) and 44 % (within 24 h of MN) in comparison to the control. Sex-specific analysis showed that the value of BI_{vn} was significantly lower in the control and within 1 h of MN in males than in females, by 20 % and 29 %, respectively, but there was no significant difference between males and females within 24 h of MN. Males and females of group 1 did not differ in BI_{ACh}. BD_{ACh} in males was less than in females only in the control (by 37 %), but not in MN.

An analysis of the indicators in animal group 2 showed that the development of MN in light deprivation in males did not cause a significant change in BI_{vn} (*Table 2*).

BI_{ACh} was decreased by 41 % (within 1 h of MN) and 37 % (within 24 h of MN). The indicator of BD_{ACh} was not changed. Female BI_{vn} was 15 % and 48 % decreased within 1 h and 24 h of MN, respectively, as compared to the control. Female BI_{ACh} of group 2 was 27 % and 53 % decreased within 1 h and 24 h of MN, respectively. There were no significant changes in BD_{ACh}. Sex-specific analysis of group 1 indicators showed that male BI_{vn} was lower than that in females, in particular, by 75 % in the control, by 73 % (within 1 h of MN) and by 17 % (within 24 h of MN). A reduction in the difference was due to this indicator changes only in females. There was no sex difference in BI_{ACh}. Male BD_{ACh} was less than that in females of group 2, by 91 % – in the control, by 44 % (within 1 h of MN), by 50 % (within 24 h of MN).

A comparative analysis of the light deprivation influence on the studied parameters between the indicators of group 1 and group 2 showed the following. There was no difference in the male cohort with regard to BI_{vn}. BI_{ACh} in males of group 2 was higher than that in animal group 1, 2.1 times in the control, 1.5 times within 1 h of MN, 1.5 times within 24 h of MN. BD_{ACh} was 46 % ($P < 0.05$) less only in the control and did not differ in MN development between male indicators of both groups. In females of group 2, BI_{vn} was 46 % higher in the controls, 18 % – within 1 h of MN, 22 % – within 24 h of MN; BI_{ACh} was 2.0 times higher in the controls, 1.8 times – within 1 h of MN; BD_{ACh} was higher by 27 % within 1 h of MN and by 13 % within 24 h of MN.

The results of analysis between indicators in animal group 3 revealed (*Table 3*) that male BI_{vn} was 1.9 times and 5.2 times decreased within 1 h and 24 h of MN, respectively; BI_{ACh} was 1.5 times and 2.6 times increased within 1 h and 24 h of MN, respectively; BD_{ACh} was also 1.5 times and 2.0 times increased within 1 h and 24 h of MN, respectively.

In females, BI_{vn} was 1.7 times increased within 1 h of MN, but it was 12.1 times decreased within 24 h of MN; BI_{ACh} was 10.3 times decreased within 1 h of MN, and it was 1.8 times less than in the control within 24 h; BD_{ACh} was also less than in the control, in particular, by 4.2 times and 1.5 times within 1 h and 24 h of MN, respectively. Sex-specific analysis of group 2 indicators showed that BI_{vn} in males was less than in females only in the control and within 1 h of MN, by 2.4 times and 7.6 times, respectively. Male BI_{ACh} in the control were 8.0 times less than in females, 2.0 times within 1 h of MN and 1.7 times within 24 h of MN. BD_{ACh} in males was 3.6 times less than in the control females, it was 1.8 times higher within 1 h of MN and 17 % less within 24 h of MN as compared to females.

Comparison of indicators between animal group 3 and group 1 showed that male BI_{vn} in group 3 was higher than that in group 1, in particular, by 6.2 times in the controls and by 3.2 times within 1 h of MN. BI_{ACh} was decreased in the control and within 1 h of MN by 2.1 times and 32 %, respectively, and 28 % increased within 24 h of MN. BD_{ACh} was 1.9 times less than in the control. Female BI_{vn} in group 3 was higher than that in group 1, in particular, by 12.2 times in the control, by 18.7 times within 1 h of MN, by 24 % within 24 h of MN. Control BI_{ACh} was 3.9 times higher than the compared value, BI_{ACh} was 2.4 times lower within 1 h of MN and 1.8 times higher within 24 h. BD_{ACh} was 39 % higher in the control, 1.9 times lower within 1 h of MN and 30 % higher within 24 h of MN.

Analysis of indicators in the animal group 4 showed (*Table 4*) that male BI_{vn} in MN was increased, in particular, by 1.5 times within 1 h of MN and by 3.0 times within 24 h of MN.

BI_{ACh} was 2.3 times increased within 1 h of MN, but this indicator did not differ from the control within 24 h of MN. BD_{ACh} in this model was not changed. Female BI_{vn} was 21 % decreased within 1 h of MN, and 28 % higher within 24 h of MN than in the control. BI_{ACh} was 37 % and 3.3 times decreased within 1 h and 24 h of MN. BD_{ACh} was not changed. Sex-specific analysis of group 4 indicators revealed that control BI_{vn} was 1.8 times less in males than that in females, there was no sex difference within 1 h of MN, and this indicator was 33 % higher in males than that in females within 24 h of MN. Male BI_{ACh} in similar conditions was 33 % less in the control than that in females, but it was 2.4 times and 1.9 times higher within 1 h and 24 h of MN, respectively. BD_{ACh} did differ significantly between males and females.

Comparison of the animal indices between group 4 and 1 indicated no difference in control BI_{vn} between males of group 4 and group 1. However, in MN development, male BI_{vn} value in group 4 was significantly higher, by 32 % and by 2.4 times within 1 h and 24 h, respectively. A similar comparison demonstrated that BI_{ACh} was 29 % and 3.0 times higher in the control and within 1 h of MN development, respectively. BD_{ACh} did not differ in the groups compared. In females of group 4, BI_{vn} was 30 % higher in the control, there was no significant difference within 1 h of MN, and it was 2.1 times higher within 24 h of MN than the value compared. Female BI_{ACh} in group 4 was 73 % and 41 % higher in the control and within 1 h of MN, respectively, than in group 1, and it was 2.2 times lower within 24 h of MN. The indices of BD_{ACh} did not differ between comparison groups.

Table 3. Indicators of the rat heart response to cholinergic stimulation in the development of myocardial necrosis in permanent lighting, M ± m

Indices	Sex	Control, n = 6	MN 1 hour, n = 6	MN 24 hours, n = 6
BI_{vn}	♂	42.18 ± 2.27[3,2]	21.86 ± 1.60[1,3,2]	8,11 ± 0,30[1]
	♀	100.72 ± 4.52[3,2]	166.36 ± 5.76 [1,3,2]	8,31 ± 0,33 [1,2]
BI_{ACh}	♂	1.91 ± 0.08[2,3]	2.95 ± 0.17[1,2,3]	5.05 ± 0.15[1,2,3]
	♀	15.30 ± 0.45 [2,3]	1.48 ± 0.10[1,2,3]	8.36 ± 0.35[1,2,3]
BD_{ACh} (sec)	♂	9.9 ± 0.5[2,3]	15.0 ± 1.7[1,3]	19.9 ± 1.2[1,3]
	♀	35.8 ± 1.8 [2,3]	8.5 ± 0.4[1,2,3]	23.2 ± 1.7[1,2,3]

[1]: significance of the differences in comparison with the control, $P < 0.05$; [2]: significance of the differences in comparison with group 1, $P < 0.05$; [3]: significance of the differences between sexes, $P < 0.05$.

Table 4. Indicators of the rat heart response to cholinergic stimulation in the development of myocardial necrosis and permanent lighting with melatonin correction, M ± m

Indices	Sex	Control, n = 6	MN 1 hour, n = 6	MN 24 hours, n = 6
BI_{vn}	♂	5.99 ± 0.26[3]	9.07 ± 0.33[1,2]	18.23 ± 0.91[1,3,2]
	♀	10.69 ± 0.39[3,2]	8.82 ± 0.33[1]	13.70 ± 0.62[1,3,2]
BI_{ACh}	♂	5.10 ± 0.29 [2,3]	11.79 ± 0.49[1,2,3]	5.00 ± 0.27 [3]
	♀	6.80 ± 0.35 [3,2]	4.95 ± 0.28[1,2,3]	2.07 ± 0.11[1,2,3]
BD_{ACh} (sec)	♂	17.4 ± 2.3	14.7 ± 2.1	14.2 ± 2.3
	♀	21.2 ± 1.7	18.3 ± 1.6	21.3 ± 3.0

[1]: significance of the differences in comparison with the control, $P < 0.05$; [2]: significance of the differences in comparison with group 1, $P < 0.05$; [3]: significance of the differences between sexes, $P < 0.05$.

Discussion

The results obtained in our studies demonstrated a light desynchronosis influence on the cholinergic heart structures functional state regardless of the variant of lighting regime violation (light deprivation or permanent lighting). A 10-day stay in the dark caused a significant increase in the sensitivity of cholinoreceptors in the sinoatrial node, which was confirmed by an increase in BI_{ACh} after ACh injection into the jugular vein. In males, this effect was impeded by a shortening of the functional desensitization period (decrease in BD_{ACh}). In contrast, the increase in

BD_{ACh} indicated a longer desensitization and a higher baseline functional activity of cholinoreceptors in the sinoatrial node in females. In this context, the development of MN was characterized by the greater sensitivity of the heart to exogenous ACh than in the light balance. It was confirmed by the greater values of BI_{ACh}. Such changes were more significant in females. Simultaneous increase in BD_{ACh} was indicative of the increase in time of functional desensitization, which also allows for longer duration of bradycardia and depends on the rate of ACh release from the synaptic cleft depending on an acetylcholinesterase influence.

The study of the animal heart response to the electrical stimulation of vagus nerve in MN development and light balance showed the pattern which was observed by other authors in the studies using adrenaline to induce MN [7]. Such data consistency validated the correctness of experimental conditions and optimal dose of adrenaline. Cardiotoxic effect of adrenaline has been confirmed biochemically [15]. Interestingly, 10-day light deprivation did not affect the rate of BI_{vn} in males, but contributed to its increase in females. The last fact was predicted by us, given that the development of MN causes more significant changes in the female activity of the heart cholinergic structures than in male [7,16,17]. The absence of changes in male BI_{vn}, which value reflects the ACh content in the presynaptic vagus nerve terminals, may indicate either a lower melatonin mediation of the resynthesis and accumulation of a neurotransmitter in the presynaptic compartment, or its smaller amount in the body. It is known that in vertebrates, the hormone is mainly synthesized in darkness [18], therefore we rather tend to think that melatonin synthesis was less in males than in females as a result of 10-day complete darkness.

The permanent lighting model yielded interesting and unpredictable results. The desynchronosis modeled in this way resulted in dramatically increased value of IB_{vn}, in particular by 6.2 times in males and by 12.2 times in females, indicating a significant accumulation of ACh in the presynaptic compartment. This fact can be explained in view of the autonomic nervous system role in the chronotropic heart function maintenance. It is known that the absence of darkness, which is the main condition for the realization of the pineal gland circadian activity, is a severe stress, a cause of sleep onset insomnia [5], a factor of coronary heart disease and arterial hypertension progression due to a high sympathoadrenal activity [11]. In this case, the phenomenon of "enhanced antagonism" of the vagus nerve may be triggered in conditions of delayed inactivation of norepinephrine. That is, the higher the adrenergic activity, the more significant is the heart response to vagus nerve stimulation, and this effect is attenuated largely by beta-adrenoceptors blockade [20]. Interestingly, in our experiment at the same time, female sensitivity of cholinoreceptors (BI_{ACh}) and duration of functional desensitization were increased. This idea is evidenced by the fact that, in our study, the female value of BI_{vn} continued to increase within 1 h of MN, which is a period of hyperadrenalinemia, despite the significantly decreased cholinoreceptor sensitivity (BI_{ACh}) and the significantly reduced time of functional desensitization. It is unlikely that accumulation of a large amount of ACh in the vagus nerve presynaptic department alone could explain such effect in the conditions of 10-day permanent lightning, since a long-term absence of darkness and normal sleep would have depleted the depot of ACh to impede constantly high adrenergic activity. In this case, the role of intestinal natriuretic peptide may be considered as a non-cholinergic component capable of mediating the effects of vagus nerve [20], or nitrogen monoxide appears to be involved, the role of which in cholinergic effects mediation is more significant in females [21]. There is evidence of greater involvement of nitrogen monoxide in mediating the heart cholinergic effects in females [22]. Unlike females, males presented the decrease in BI_{vn} while cholinoreceptor sensitivity (BI_{ACh}) and the time of functional desensitization (BD_{ACh}) increasing within 1 h of MN development in the permanent lightning, which could be the result of faster synergistic desensitization [20] and was evidenced by a continuation of this trend within 24 h of MN (worsening of damage signs) in animals of both sexes. Generally, males and females demonstrated opposite reactions in conditions of hyperadrenalinemia, which indirectly reflects not only different levels of melatonin deficiency, but also different patterns of cardiovascular system adaptation in pathological conditions.

Based on the data obtained, to confirm the pineal hormone deficiency in conditions of permanent lightning and for cardioprotection, exogenous melatonin was used in animal group 4. It is known that the maximum level of hormone in the mediobasal hypothalamus, striatum and neocortex occurs in an hour after its parenteral injection, and a sufficiently high level is maintained in blood plasma for 3–4 hours. The positive effect of melatonin has been established in the treatment of patients with myocardial infarction, after angioplasty to prevent reperfusion injury [23,24]. The results obtained have confirmed a melatonin deficiency in conditions of permanent lightning in animals as all the indicators reflecting the heart response to cholinergic stimulation differed from those in animal group 3. The comparison of absolute values showed a tendency to approximation (but not restoration) of all the cholinoreceptor state parameters characterizing the light balance. In our opinion, exogenous melatonin served as an anti-stress factor that reduced the adrenergic reactivity and effect of "enhanced antagonism" [19]. In the development of MN, the dynamics of BI_{vn}, BI_{ACh}, and BD_{ACh} were different from those in animal group 1. That is, in our study, exogenous melatonin most likely displayed a regulatory function that is realized involving two mechanisms – cAMP and cGMP-PKG (protein kinase G) – dependent pathways [8]. The final effect was indicative of not only the more balanced heart response to cholinergic stimuli, but also the decrease in adrenoreactivity, which was confirmed by the more significant dynamics of indicators in males of group 4 as well as data from previous studies, which had demonstrated the higher myocardial irritability related to the damaging effect of adrenaline in males [15].

Conclusions

1. 10-day light deprivation increases the response of rat heart to cholinergic effects maintaining such state

in conditions of adrenaline-induced myocardial necrosis, and is realized by increased cholinoreceptor sensitivity and acetylcholine accumulation in the vagus nerve presynaptic department in females and only by increased cholinoreceptor sensitivity in males.

2. 10-day permanent lightning (500 lux) causes the substantial increase in the heart response to cholinergic stimulation. The more intensive response of the female heart to vagus nerve stimulation, compared with male, is caused by the increased release of acetylcholine from the presynaptic compartment with simultaneous increase in the cholinoreceptor sensitivity, in contrast to decreased male cholinoreceptor sensitivity. The development of adrenaline-induced myocardial necrosis in females is characterized by greater sensitivity of the heart cholinergic structures to functional influences compared to light balance conditions and male response.

3. Melatonin (5 mg/kg) reduces the heart response to cholinergic stimulation in conditions of permanent lightning, results in the approximation (but not restoration) of the intensity of the heart response to cholinergic stimuli, which is characteristic of light balance conditions. At the same time, the sensitivity of cholinoreceptors is greater than in conditions of light balance, especially in females. The development of adrenaline-induced myocardial necrosis in this case is characterized by greater heart response to stimulation of vagus nerve than in light balance regardless of sex, and is combined with greater cholinoreceptor sensitivity in males.

The prospect of further research includes detecting the sex differences in melatonin metabolism (endogenous and exogenous) in adrenaline-induced myocardial necrosis and light desynchronosis, which is relevant to the gender pharmacology development.

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