ORIGINAL ARTICLE

CONTENTS

Changes in autonomic balance of heart rate in male rats subjected to hypodynamia stress and gonadectomy

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ABSTRACT

Aim: To analyze changes in variational cardiointervalometry indicators in male rats that have undergone castration and stress and their combination.

Materials and Methods: The research was carried out on 48 white sexually mature rats, aged from 1.5 to 3.0 months, weighing from 180 to 200 g. All animals were divided into 4 experimental group: 1 – control, 2 – gonadectomy, 3 – stress, 4 – gonadectomy and stress. To study heart rate variability, the method of variational cardiointervalometry was used on "Cardiolab" (Kharkiv, Ukraine). ECG analysis imclude calculation of heart rate (HR, min-1); variational range of cardiac intervals (ΔX, s); mode (Mo, s); amplitude of mode (AMo, %); tension index (TI); index of autonomic balance; autonomic rhythm index; indicator of adequacy of regulatory processes. ECG recording was performed under thiopental sodium anesthesia.

Results: Mo decreased in group 2 of rats, compared to 1, by 9.9 % ($p<0.01$). It was lower in group 3, compared to 1, by 11.2 % ($p<0.001$) and – group 4 – by 8.7 % ($p<0.01$). AMo decreased, compared to the control, only in group 3 by 22.4 % ($p<0.001$) and was lower compared to groups 2, and 4. The lowest ΔX values were observed in group 2 of rats. HR increased in group 2 of rats, compared to group 1, by 11.0 % ($p<0.001$), in group 3 of rats, compared to group 1, by 12.7 % ($p<0.001$) and was higher in this group, compared to group 4, by 8.0 % ($p<0.01$). Increase in the TI was noted in group 2 of rats, compared to group 1 of rats, by 2.5 times ($p<0.001$), in group 3 of rats, compared to group 1, – by 2.1 times ($p<0.001$), in group 4 of rats – by 88.7 % ($p<0.001$).

Conclusions: A difference in the provision of autonomous regulation of heart rate was found in animals that had been stressed and castrated, which can cause the greatest damage to the heart with gonadectomy and stress.

KEY WORDS: gonadectomy, rats, stress, regulatoy mechanism, heart rhythm

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INTRODUCTION

The effects of eustress and distress on the body have been studied for many years [1]. Due to the effects of prolonged stress on the body of people around the world, due to the unstable economic and political situation, a large number of people are exposed to excessive stress. One of the systems that reacts most to its effects is the cardiovascular system. Diseases associated with it continue to lead the world [2, 3]. Another problem today is physical inactivity, which is also one of the risk factors for the development of cardiovascular diseases [4]. Stress in animals causes inhibition of testosterone synthesis and spermatogenesis due to the blockade of gonadotropin-releasing hormone receptors and a decrease in the release of luteinizing and follicle-stimulating hormones, which leads to the cessation of testosterone secretion and gametogenesis [5]. Both factors cause male infertility. It is obvious that such influences will disrupt regulatory processes, in particular on the part of the autonomic nervous system.

Preclinical studies indicate a complex effect of testosterone on the risk of cardiovascular disease through effects on cardiomyocytes and the vascular system. Moreover, low testosterone levels have a causal relationship with the development of pathological metabolic changes in the myocardium. Studies have shown worse metabolic profiles in men with organic hypogonadism. However, a consistent association between serious cardiovascular events and male hypogonadism has not been established [6].

Testosterone has been shown to have vasodilatory properties, which are more pronounced in the short term due to non-genomic action [7]. Despite the known endothelium-dependent vasodilatory effects of testosterone through inhibition of voltage-gated calcium channels [8], its vasoconstrictor effects have

also been discovered [9, 10]. The genomic action of testosterone has been associated with vasoconstriction through adrenergic effects on vascular smooth myocytes [11]. In addition, androgens promote vascular calcification, the release of reactive oxygen species and hypertrophy [7]. It has been shown that the interaction between the genomic and non-genomic actions of testosterone affects the functions of the cardiovascular system [7], directly or indirectly affecting the myocardial oxygen demand and the ability to supply the myocardium with this oxygen. It has also been shown that testosterone activates mitochondrial ATP-sensitive potassium channels, which leads to increased oxidation of flavoproteins in mitochondria, reduces oxidative stress levels by reducing malondialdehyde levels and increasing the activity of superoxide dismutase and glutathione peroxidase, and increases the expression of factors PGC-1α, NRF-1, and TFAM, which supports mitochondrial function [12, 13].

There is a common effect on the myocardium through the activation of β_2-receptors under the action of the sympathetic nervous system, moderately elevated concentrations of adrenaline during chronic stress, which leads to an increased myocardial oxygen demand. However, distinct effects are also noted. Thus, during activation of the sympathetic nervous system, vasodilation of coronary vessels occurs due to the activation of β_2-receptors, which is less pronounced in chronic stress due to the depletion of β_2-receptors. During acute stress, α_1-receptors are activated, which, on the contrary, contributes to vasoconstriction of coronary vessels [14-17].

Thus, taking into account the predominantly short-term non-genomic and long-term genomic effects of testosterone, as well as the variable duration of action of catecholamines (depending on the type of stress) and the sympathetic nervous system, one can expect differences in the nature of changes in the regulatory mechanisms of the cardiovascular system.

In particular, the pathophysiological mechanisms involved in hypogonadism, stress, and their combination are different in nature and clinical consequences.

This emphasizes the feasibility of studies aimed at detailing the impact of androgen deficiency and stress factors on the regulatory mechanisms of the cardiovascular system.

AIM

The aim of the study was to analyze changes in variational cardiointervalometry indicators in male rats that have undergone castration and stress and their combination.

MATERIALS AND METHODS

The work was doing at the Central Research Laboratory of Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine.

All experiments were performed at the first part of day in a specially designated room at illumination of 250 lux, a temperature of 18-22 °C and relative humidity of 40-60%. Animals were kept and experiments on them in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes. The Commission on Bioethics of Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (protocol No 80 from January 10, 2025) did not find any violations of ethical norms during the conduct of research work.

The research was carried out on 48 white sexually mature rats, aged from 1.5 to 3.0 months, weighing from 180 to 200 g. All animals were divided into 4 experimental group: 1 – control, 2 – gonadectomy, 3 – stress, 4 – gonadectomy and stress (Table 1).

Stress was induced in rats from 1.5 to 3 months of age. The animals were constantly kept in cages with limited living space for 1.5 months [18].

Experimental modeling of the reduction of sex hormone levels in rats was carried out by castration under thiopental-sodium anesthesia (40 mg/kg) surgically according to the method of J. D. Kirshenblatt through a median incision of the anterior surface of the skin of the scrotum, where the testicle is located [19, 20]. The age of the rats that underwent gonadectomy was 1.5 months.

In 4-th group of rats, hypogonadism was first simulated, and then hypodynamia.

To study heart rate variability, the method of variational cardiointervalometry was used [21]. For this purpose, the device "Cardiolab" (Kharkiv, Ukraine) was used. 1000 R–R cardiac intervals were recorded. Using a computer program, ECG analysis was performed and calculation of heart rate (HR, min-1); variational range of cardiac intervals (ΔX, s); mode (Mo, s); amplitude of mode (AMo, %); tension index (TI=AMo/(2·ΔX·Mo)); index of autonomic balance (IAB=AMo/ΔX); autonomic rhythm index (ARI=1/(Mo·ΔX)); indicator of adequacy of regulatory processes (IARP=AMo/Mo). ECG recording was performed under thiopental sodium anesthesia (40 mg x kg-1 of animal body weight intraperitoneally).

The reliability of the obtained differences between the results (minimum significance level $p<0.05$) was assessed using the Kruskal–Wallis and Newman–Keuls criteria (BioStat program, AnalystSoft Inc).

Rats were housed in standard vivarium conditions with free access to water and food.

Table 1. Design of experiment – division of experimental animals

Group number	Investigation condition			Quantity of animal
	Gonadectomy	Stress	Gonadectomy + stress	
I	-	-	-	12
II	+	-	-	12
III	-	+	-	12
IV	-	-	+	12
Total				48

Table 2. Changes in the indicators of mathematical analysis of heart rate in rats under the influence of hypodynamia and hypogonadism, (M ± σ, n=12)

Group	Index			
	Mo, sec	AMo, %	ΔX, sec	HR
Group 1 – Control				
Control	0.1379 ± 0.0046	35.2 ± 1.4	0,0048 ± 0,0009	435.01 ± 17.03
Group 2 – Gonadectomy				
Gonadectomy	0.1243 ± 0.0047 #	35.8 ± 2.2	0,0038 ± 0,0001 #	482.70 ± 16.21 #
Group 3 – Stress				
Stress	0.1224 ± 0.0025 #	27.3 ± 1.6 #,##	0,0062 ± 0,0007 ##	490.20 ± 10.14 #
Group 4 – Gonadectomy + stress				
Gonadectomy + stress	0.1331 ± 0.0051 ###	32.6 ± 3.8 ###	0,0061 ± 0,0009 ##	450.79 ± 18.03 ###

Note: # – probable differences with the corresponding term of series 1;
– probable differences with the corresponding term of series 2;
– probable differences with the corresponding term of series 3

RESULTS

When analyzing the variation cardiointervalometry indicators (Table 2), the following was noted in all four groups.

The value of Mo decreased in group 2 of rats, compared to group 1, by 9.9 % ($p<0.01$). It was also lower in group 3 of rats, compared to group 1, by 11.2 % ($p<0.001$) and was lower compared to group 4 of animals by 8.7 % ($p<0.01$). It can be thought that both gonadctomy and stress cause an increase in the secretion of catecholamines by the adrenal glands, although the combined pathology practically does not affect this process, i.e. causes adaptation to stress.

AMo significantly decreased, compared to the control, only in group 3 of rats by 22.4 % ($p<0.001$) and was lower in this group, compared to group 2, by 23.7 % ($p<0.001$) and in group 4 – by 16.3 % ($p<0.001$). It is obvious that in hypodynamia, in response to the increase in the release of catecholamines by the adrenal glands, there is a compensatory decrease in the release of catecholamines by nerve terminals. Such changes may indicate a greater reaction of the autonomic nervous system precisely during castration.

The lowest ΔX values were observed in group 2 of rats: compared to group 1 by 20.8 % ($p<0.001$), group 3 – by 84.2 % ($p<0.001$), group 4 – by 60.5 % ($p<0.001$). The decrease in the tone of the parasympathetic division of the autonomic nervous system can also be considered a compensatory reaction to gonadectomy with increased adrenaline secretion by the adrenal glands.

Heart rate increased in group 2 of rats, compared to group 1, by 11.0 % ($p<0.001$), in group 3 of rats, compared to group 1, by 12.7 % ($p<0.001$) and was higher in this group, compared to group 4, by 8.0 % ($p<0.01$). Heart rate is directly related to the value of Mo; as the latter increases, it decreases, and as Mo decreases, it increases.

Analysis of calculated indicators is presented in Table 3.

At the same time, an increase in the TI of regulatory mechanisms was noted in group 2 of rats, compared to group 1 of rats, by 2.5 times ($p<0.001$), in group 3 of rats, compared to group 1, – by 2.1 times ($p<0.001$), in group 4 of rats – by 88.7 % ($p<0.001$). Obviously, gonadectomy causes an increase in the tension of regulatory mechanisms.

At the same time, an increase in IAB was noted in group 2 of rats, compared with group 1, by 28.5 % ($p<0.001$), in group 3 of rats – by 53.3 % ($p<0.001$), in group 4 of rats – by 43.3 % ($p<0.001$). In group 3 of rats, the result was the smallest: compared with group 1 of rats by 40.0 % ($p<0.001$), with group 4 of rats – by 21.4 % ($p<0.001$). In group 4 of rats, the indicator was lower compared with group 1 of rats by 27.1 % ($p<0.001$).

Table 3. Changes in calculated indicators of mathematical analysis of heart rate in rats with hypodynamia and hypogonadism, (M ± σ, n=10)

Group	Index			
	TI, x10³, Units	IAB, Units	ARI, Units	IARP, Units
Group 1 – Control				
Control	15.11 ± 2.01	7333 ± 246	1511 ± 318	0,255 ± 0,011
Group 2 – Gonadectomy				
Gonadectomy	37.90 ± 3.37 #	9421 ± 685 #	2117 ± 112 #	0,288 ± 0,021 #
Group 3 – Stress				
Stress	17.99 ± 2.28 ##	4403 ± 452 #,##	1318 ± 148 ##	0,223 ± 0,016 #,##
Group 4 – Gonadectomy + stress				
Gonadectomy + stress	20,08 ± 6,02 ##	5344 ± 348 #,###	1232 ± 254 ##	0,245 ± 0,022 ##

Note: # – probable differences with the corresponding term of series 1;
– probable differences with the corresponding term of series 2;
– probable differences with the corresponding term of series 3

ARI increased the most in 2 group of rats, compared to 1 group of rats, by 40.1 % ($p<0.001$), to the 3 group of rats – by 37.7 % ($p<0.001$), to the 4 group of rats – by 41.8 % ($p<0.001$).

IARP increased the most in 2 group of rats, compared to 1 group of rats, by 12.9 % ($p<0.01$), to the 3 group of rats – by 22.6 % ($p<0.001$), to the 4 group of rats – by 14.9 % ($p<0.001$). Also in 3 group of rats, compared to 1 one, the indicator was decrased by 8.6 % ($p<0.05$).

The obtained data indicate the greatest predominance of the tone of the sympathetic division of the autonomic nervous system in animals of group 2 of rats.

DISCUSSION

The literature contains data on the disclosure of mechanisms of damage to the cardiovascular system in various pathological processes, which is associated with endocrine regulation [22, 23], prolonged hypodynamia [24], thermal trauma [25]. This problem is especially important among male patients with oncological pathology – prostate cancer [26], but there is practically no data on regulatory mechanisms that ensure the processes of adaptation of the cardiovascular system to pathological conditions.

The changes we have shown, which concern gonadectomized rats, indicate an increase in the release of catecholamines by the adrenal glands, a decrease in cholinergic protective effects, and this causes a significant tension of regulatory mechanisms, which has a harmful effect on the cardiovascular system. First, the development of arrhythmias increases, and secondly, metabolic processes, which will contribute to a greater need for oxygen [24]. New data indicate that sympathetic regulation contributes to the progression of prostate cancer [28]. Such data require further study, since gonadectomy is the most important method in the treatment of prostate cancer [26], but the hypersympathicotonia that occurs in this case can provoke a deterioration in the condition of patients. Therefore, attention should be paid to increasing the tone of the parasympathetic division of the autonomic nervous system.

In rats that have undergone prolonged hypodynamic stress, an increase in the release of catecholamines by the adrenal glands was noted, but a decrease in the release of noradrenaline by nerve terminals, there is a tendency to increase the tone of the parasympathetic division of the autonomic nervous system. If the latter is considered as a compensatory mechanism that has an oxygen-saving effect, then this is one of the most appropriate mechanisms in conditions of increased heart rate. Such data are natural, lead to complications and are confirmed by the data of the literature [29, 30].

The mechanism of normalization of cardiointervalogram indicators in animals that have undergone combined pathology becomes unclear. In them, except for the lower IAB, all indicators do not differ from the control ones. It is impossible to prescribe to patients with gonadectomy to lead a sedentary lifestyle, because with hypodynamia sympathetic activity also increases, therefore, the next steps may be studies of cholinergic regulation of cardiac activity. We have not found in the literature mechanisms of such a paradoxical, as it seems, reaction.

CONCLUSIONS

A difference in the provision of autonomous regulation of heart rate was found in animals that had been stressed and castrated, which can cause the greatest damage to the heart with gonadectomy and stress.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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