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CHANGES OF ELECTROCARDIOGRAMS OF ANIMALS WITH EPINEPHRINE HEART DAMAGE IN INFLUENCES OF STRESS AND CASTRATION

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Abstract

The study of changes in electrocardiograms under the influence of stress on the body is relevant. The catecholamines released in this case cause disorders of the work of the heart and contribute to the development of cardiovascular pathology. Stress also causes suppression of testosterone synthesis and spermatogenesis.

The aim of the study was - to analyze changes in electrocardiogram indicators in rats that have undergone castration and stress during the development of epinephrine heart damage (EHD).

Material and methods of investigation. The study was performed on 240 white Wistar male rats, which were kept in one room on a standard diet and a vivarium regime. All animals were divided into four series: 1 – control, 2 – stress, 3 – castration, 4 – castration and stress. To simulated EHD, rats were injected once intramuscularly with a 0.18% solution of adrenaline hydrotartrate at the rate of 0.5 mg/kg of rat's weight. Such a dose of adrenaline causes reliable regulatory changes in the functioning of the cardiovascular system under any environmental conditions already 1 hour after injection of the drug, without causing lethality among animals. Stress was induced in rats from 1.5 to 3 months of age. The animals were constantly kept in cages with the limitation of living space by two times for 1.5 months.

Experimental modeling of the decrease in the level of sex hormones in rats was carried out using castration under sodium thiopental anesthesia (40 mg/kg). Autopsy of the anterior abdominal wall was made according to Ya.D. Kirshenblatt method.

At the time of the beginning of EHD simulation, all animals were 4 months old. Electrocardiography (ECG) was made after 1, 3, 7, 14 and 28 days after injection of epinephrine hydrotartrate in volumes corresponding to body weight. ECG was made under thiopental-sodium anesthesia.

For analysis, we took the durations of the PQ and QT intervals, the status of the ST segment from the isoline, and the amplitude of the T wave. The significance of the obtained differences between the results (minimum level of significance $p<0.05$) was assessed using the Kruskal–Wallis and Newman–Keuls tests (BioStat program, AnalystSoft Inc.).

Results. When analyzing electrocardiogram indicators in the control groups of all four series, the following was noted. Analyzing T wave data, only a significantly greater amplitude of the T wave was noted in the 4th series of rats, compared to the 3rd, by 64.8% ($p<0.001$).

In 1 series of animals, during the development of EHD after 3 days, significantly higher values of the duration of the PQ interval were noted, compared to the control by 28.0% ($p<0.01$), and compared to the previous period of the study by 34.3% ($p<0.01$). Also, at this time, the amplitude of the T wave, compared to the previous period of the study, was 2.3 times higher ($p<0.001$).

In the 2nd series of animals, the amplitude of the T wave 7 days after the start of adrenaline injection was 84.3% greater ($p<0.001$) compared to the control. Also, after 28 days, compared to the previous period of the study, the amplitude of the T wave was smaller by 49.3% ($p<0.01$). When comparing the indicators with 1 series of rats, the following was noted. After 3 days, the duration of the PQ interval was shorter by 24.7% ($p<0.01$). The duration of the QT interval was not significantly different between animal series. Deviation of the ST segment from the isoline after 1 day of EHD was 76.2% ($p<0.001$) greater in 2 series of rats. The amplitude of the T wave in the 2nd series of rats, compared to the 1st, was 2.4 times greater after 3 days ($p<0.001$), after 7 days by 58.3% ($p<0.001$), and after 28 days it was smaller by 37.6% ($p <0.01$).

In the 3rd series of animals, no significant changes in indicators were noted during the experiment, compared to the control series. When the indicators of 3 series of rats were compared with ones of 1 series, it was noted that after 3 days the duration of the PQ interval was shorter by 34.3% ($p<0.01$). When comparing the indicators of the 3rd series with the 2nd series of animals, the following was noted. 1 day after the injection of adrenaline, the deviation of the ST segment from the isoline was greater in animals that experienced long-term stress by 75.7% ($p<0.001$), and the T wave amplitude by 43.8% ($p<0.001$). 7 days after the injection of adrenaline, the amplitude of the T wave was greater in animals that experienced long-term stress by 34.1% ($p<0.001$). 14 days after the injection of adrenaline, the deviation of the ST segment from the isoline was greater in animals that experienced long-term stress by 96.7% ($p<0.001$), and the amplitude of the T wave by 45.8% ($p<0.001$).

In the 4 series of animals, no significant changes in indicators were noted during the experiment, compared to the control series. Comparing the indicators of 4 series of rats with 1 series, it was noted that 1 day after the injection of adrenaline, the amplitude of the T wave was 2.5 times higher in 4 series of rats ($p<0.001$); after 3 days, the duration of the PQ interval was shorter by 24.7% ($p<0.01$). When comparing the indicators of the 4th series with the 2nd series of animals, the following was noted. 1 day after the injection of adrenaline, the deviation of the ST segment from the isoline was greater in animals that subjected to long-term stress by 64.9% ($p<0.001$), and after 3 days – by 69.5% ($p<0.001$). 7 days after the injection of adrenaline, the amplitude of the T wave was greater in animals that subjected to long-term stress by 43.8% ($p<0.001$). 28 days after the injection of adrenaline, the duration of

the QT interval was longer in animals that underwent castration and stress by 17.8% ($p<0.05$). When comparing the indicators of 4 and 3 series of rats, it was noted that 1 day after the injection of adrenaline, the amplitude of the T wave was higher in 4 series of rats by 85.0% ($p<0.001$).

Conclusions. The difference in the most vulnerable periods of damage to the myocardium by adrenaline was revealed in the studied groups of rats. For animals that are in normal conditions - after 3 days; for animals that lead a sedentary lifestyle - up to 7 days. Animals that underwent castration and animals that underwent castration and stress did not undergo reliable changes in the studied indicators of electrocardiograms.

Key words: electrocardiogram; adrenaline; heart; stress; castration

Introduction. The effect of eustress and distress on the body has been studied for many decades [1]. Due to the current events in the world, a large number of people are exposed not only to physiological stress, but also to the stress of excessive force. One of the systems most responsive to stress is the cardiovascular system. Diseases associated with it continue to lead the world [2, 3]. To study this pathology in the experiment, a model of catecholamine damage is widely used [4, 5, 6]. Another problem today is hypodynamia, which is also one of the risk factors for the development of diseases of the cardiovascular system [7]. To date, the number of patients with COVID-19 continues to be at a high level (although a milder course of the disease is noted), which can affect spermatogenesis disorders [8, 9]. Stress in animals causes suppression of testosterone synthesis and spermatogenesis in connection with the blockade of gonadoliberin receptors and a decrease in the release of luteinizing and follicle-stimulating hormones, which leads to the cessation of testosterone secretion and gametogenesis [10]. Both factors cause male infertility. There is a close relationship between hypogonadism, testosterone deficiency, and the development of cardiovascular diseases [11, 12, 13]. It is obvious that regulatory processes will be disturbed under such influences, in particular on the part of the autonomic nervous system, which will be reflected on electrocardiograms.

The aim of the study was to analyze changes in electrocardiogram indicators in rats that underwent castration and stress during the development of epinephrine heart damage (EHD).

Materials and methods of investigation. The study was performed on 240 white male Wistar rats, which were kept in one room on a standard diet and a vivarium regime. All animals were divided into four series: 1 – control, 2 – stress, 3 – castration, 4 – castration and stress. To reproduce EHD, rats were injected once intramuscularly with a 0.18% solution of adrenaline hydrotartrate at the rate of 0.5 mg/kg of weight (Pharmaceutical company "Darnytsia", Ukraine) [9]. Such a dose of adrenaline causes reliable regulatory changes in the functioning of the cardiovascular system under any environmental conditions already 1 hour after administration of the drug, without causing lethality among animals.

Stress was induced in rats from 1.5 to 3 months of age. The animals were constantly kept in cages with the limitation of living space by two times for 1.5 months [10].

At the time of the beginning of EHD reproduction, all animals were 4 months old, after injection of epinephrine hydrotartrate in volumes corresponding to body weight, after 1,

3, 7, 14 and 28 days, under thiopental-sodium anesthesia, ECG was recorded. Experimental modeling of a decrease in the level of sex hormones in rats was carried out using castration under sodium thiopental anesthesia (40 mg/kg). Autopsy of the anterior abdominal wall was made according to Ya.D. Kirshenblatt method [11, 12].

All experiments were done in the first half of the day at a temperature of 18-22°C, relative humidity of 40-60% and illumination of 250 lux. The experiments were performed in compliance with the norms of the Council of Europe Convention on the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, March 18, 1986), the resolution of the First National Congress on Bioethics (Kyiv, 2001) and the order of the Ministry of Health of Ukraine No. 690 dated September 23.2009.

The device "Cardiolab" (Kharkiv, Ukraine) was used to study electrocardiograms. ECG recording was performed under sodium thiopental anesthesia (40 $mg \cdot kg^{-1}$ of the animal's body weight intraperitoneally).

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

Results and discussion. For the analysis, we took the durations of the PQ and QT intervals, the deviation of the ST segment from the isoline, and the amplitude of the T wave. When analyzing the electrocardiogram indicators (table 1) in the control groups of all four series, the following was noted. Of the parameters analyzed by us, only a significantly greater amplitude of the T wave was noted in the 4th series of rats, compared to the 3rd ones, by 64.8% ($p<0.001$). Such data may indicate the development of myocardial hypoxia in animals that have undergone castration and stress. Despite the fact that the deviation of the ST segment from the isoline in 2 series of rats, compared to 1 series, was 4.8 times greater, in 3 – by 82.6%, and in 4 – by 2.8 times, there were no significant differences between the groups. It is obvious that the development of dystrophic processes occurred to a different extent in animals within the same series, and rats that were in a state of hypodynamia for a long time underwent the greatest changes, and castration reduced the development of dystrophic processes in animals, but did not prevent disruption of metabolic processes in the myocardium.

In 1 series of animals, during the development of EHD after 3 days, significantly higher values of the duration of the PQ interval were noted, compared to the control by 28.0% ($p<0.01$), and compared to the previous period of the study by 34.3% ($p<0.01$). Such changes indicate the slowing down of impulses by the atria and may indicate a vulnerable period and the threat of arrhythmias. Also, at this time, the amplitude of the T wave, compared to the previous period of the study, was 2.3 times higher ($p<0.001$), which indicates the development of myocardial hypoxia. Deviations of the ST segment from the isoline were greater at all times of the study, but again the results were not significantly different.

In the 2nd series of animals, the amplitude of the T wave 7 days after the start of adrenaline injection, compared to the control, was greater by 84.3% ($p<0.001$), which indicates the development of myocardial hypoxia. Also, after 28 days of adrenaline injection, compared to the previous period of the study, the amplitude of the T wave was smaller by 49.3% ($p<0.01$). When comparing the indicators with 1 series of rats, the following was noted. After 3 days, the duration of the PQ interval was shorter by 24.7% ($p<0.01$), which indicated the normal conduction of impulses by the atria in 2 series of rats. The duration of the QT

interval was not significantly different between animal series. The deviation of the ST segment from the isoline after 1 day of EHD was 76.2% ($p<0.001$) greater in 2 series of rats, which indicated the development of metabolic disorders in the myocardium of animals that led a sedentary lifestyle. The amplitude of the T wave in the 2nd series of rats, compared to the 1st, was greater after 3 days by 2.4 times ($p<0.001$), after 7 days by 58.3% ($p<0.001$), which indicated the development of myocardial hypoxia, and after 28 days it turned out to be smaller by 37.6% ($p<0.01$).

Table 1 – Changes in electrocardiogram indicators in rats during the development of adrenaline damage to the heart, ($M \pm \sigma$, n=10)

Group	Indicator			
	PQ, ms	QT, ms	ST, mV	T, mV
Series 1 – Control				
Control (Intact)	45.90 ± 1.91	62.75 ± 9.16	0.023 ± 0.050	0.126 ± 0.049
1 day EHD	43.75 ± 1.77	61.25 ± 5.03	0.063 ± 0.028	0.073 ± 0.025
3 days EHD	58.75 ± 5.56*,**	61.00 ± 5.30	0.077 ± 0.041	0.166 ± 0.042**
7 days EHD	45.74 ± 4.85**	62.25 ± 5.58	0.087 ± 0.069	0.163 ± 0.035
14 days EHD	45.50 ± 1.58	61.55 ± 4.61	0.065 ± 0.095	0.147 ± 0.039
28 days EHD	49.25 ± 5.01	64.25 ± 3.92	0.040 ± 0.057	0.165 ± 0.058
Series 2 – Stress				
Control (Stress)	47.00 ± 2.84	57.00 ± 3.69	0.110 ± 0.052	0.140 ± 0.054
1 day EHD	43.50 ± 4.59	58.00 ± 2.84	0.111 ± 0.019$^{\#}$	0.178 ± 0.040$^{\#}$
3 days EHD	44.25 ± 3.34$^{\#}$	58.75 ± 4.75	0.131 ± 0.026	0.218 ± 0.060
7 days EHD	49.25 ± 4.42	66.00 ± 7.66	0.102 ± 0.027	0.258 ± 0.029$^{*,\#}$
14 days EHD	45.25 ± 5.46	66.25 ± 9.66	0.121 ± 0.027	0.203 ± 0.056
28 days EHD	43.25 ± 2.37	56.25 ± 4.45	0.019 ± 0.068	0.103 ± 0.042**
Series 3 – Castration				
Control (Castration)	47.75 ± 5.92	61.75 ± 3.74	0.042 ± 0.031	0.108 ± 0.029
1 day EHD	43.75 ± 2.43	61.50 ± 6.69	0.027 ± 0.029$^{\#\#}$	0.100 ± 0.020$^{\#\#}$

3 days EHD	43.75 ± 2.43#	62.00 ± 4.83	0.064 ± 0.051	0.158 ± 0.049
7 days EHD	45.25 ± 2.99	56.00 ± 6.89	0.057 ± 0.047	0.170 ± 0.020##
14 days EHD	48.75 ± 2.70	69.25 ± 3.55	0.004 ± 0.032##	0.110 ± 0.027##
28 days EHD	46.25 ± 2.12	68.00 ± 7.25##	0.031 ± 0.040	0.097 ± 0.022
Series 4 – Castration + Stress				
Control (Castration + Stress)	46.00 ± 3.37	61.50 ± 6.37	0.064 ± 0.057	0.178 ± 0.040###
1 day EHD	45.25 ± 2.99	72.25 ± 8.78	0.039 ± 0.017##	0.185 ± 0.053#,###
3 days EHD	44.25 ± 3.74#	68.25 ± 7.55	0.040 ± 0.025##	0.148 ± 0.032
7 days EHD	51.00 ± 2.69	63.00 ± 8.40	0.053 ± 0.052	0.145 ± 0.037##
14 days EHD	49.25 ± 2.65	62.75 ± 5.95	0.057 ± 0.062	0.143 ± 0.024
28 days EHD	44.25 ± 1.21	66.25 ± 5.43##	0.032 ± 0.053	0.133 ± 0.026
Notes: 1. * – probable differences with the control within the series; ** – probable differences with the results of the previous term of the study within the series; # – 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.				

In the 3rd series of animals, no significant changes in indicators were noted during the experiment, compared to the control series. When comparing the indicators of 3 series with 1 series of rats, it was noted that after 3 days after adrenaline hydrotartrate injection the duration of the PQ interval was shorter by 34.3% ($p<0.01$), which indicates normal conduction of impulses by the atria. When comparing the indicators of the 3rd series with the 2nd series of animals, the following was noted. 1 day after the injection of adrenaline, the deviation of the ST segment from the isoline was greater in animals that experienced long-term stress by 75.7% ($p<0.001$), and the T wave amplitude by 43.8% ($p<0.001$). 7 days after the injection of adrenaline, the amplitude of the T wave was greater in animals that experienced long-term stress by 34.1% ($p<0.001$). 14 days after the injection of adrenaline, the deviation of the ST segment from the isoline was greater in animals that experienced long-term stress by 96.7% ($p<0.001$), and the amplitude of the T wave by 45.8% ($p<0.001$). Such data indicate that the development of EHD causes less damage to the heart muscle of castrated rats than stress.

In the 4 series of animals, no significant changes in indicators were noted during the experiment, compared to the control series. When comparing the indicators of 4 series and 1 series of rats, it was noted that 1 day after the injection of adrenaline, the amplitude of the T wave was 2.5 times higher in 4 series of rats ($p<0.001$), which indicated hypoxic changes in

the myocardium; after 3 days, the duration of the PQ interval was shorter by 24.7% ($p<0.01$), which indicates normal conduction of impulses by the atria. When comparing the indicators of the 4th series with the 2nd series of animals, the following was noted. 1 day after the injection of adrenaline, the deviation of the ST segment from the isoline was greater in animals that experienced a long time of stress by 64.9% ($p<0.001$), and after 3 days - by 69.5% ($p<0.001$), which indicated a smaller development dystrophic processes in animals that were castrated before stress. 7 days after the introduction of adrenaline, the amplitude of the T wave was greater in animals that experienced long-term stress by 43.8% ($p<0.001$), which also indicated a lower development of myocardial hypoxia in animals that were castrated before stress. 28 days after the injection of adrenaline, the duration of the QT interval was longer in animals that underwent castration and stress by 17.8% ($p<0.05$), which indicates a violation of conduction of impulses by the ventricles and a threat of the development of ventricular arrhythmias. When comparing the indicators of 4 and 3 series of rats, it was noted that 1 day after the injection of adrenaline, the amplitude of the T wave was higher in 4 series of rats by 85.0% ($p<0.001$), which indicated hypoxic changes in the myocardium. Such data indicate that the development of EHD causes less damage to the myocardium of castrated rats before stress than after stress alone, but more damage to the myocardium of rats subjected to castration and stress than to stress alone.

Despite the absence of a significant difference in the deviation of the ST segment from the isoline, the changes were quite significant (Figure 1). The most significant changes were in the group that experienced stress 7 days after the initial injection of adrenaline.

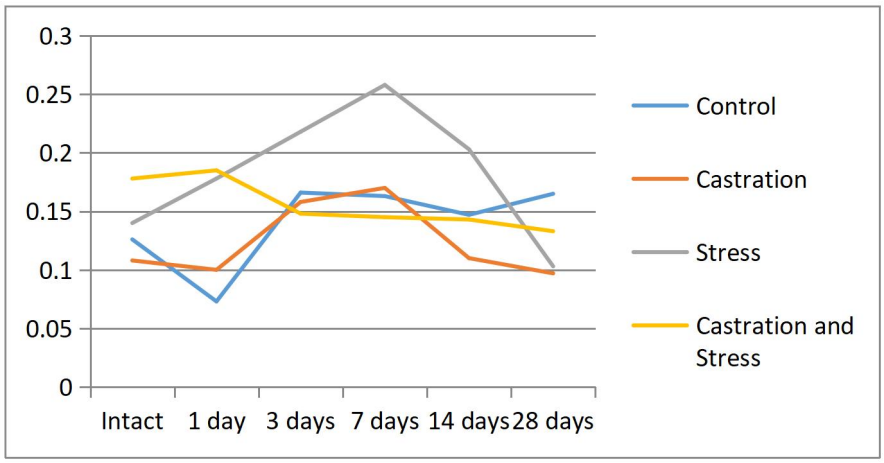

Figure 1. Deviations of the ST segment from the isoline on the electrocardiograms of rats during the development of EHD.

Thus, a difference in bioelectrical processes was found in animals that were previously subjected to long-term hypodynamic stress and castration during the development of EHD.

Conclusions. The difference in the most vulnerable periods of damage to the myocardium by adrenaline was revealed in the studied groups of rats. For animals that are in normal conditions - after 3 days; for animals that lead a sedentary lifestyle - up to 7 days. Animals that underwent castration and animals that underwent castration and stress did not undergo reliable changes in the studied indicators of electrocardiograms.

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