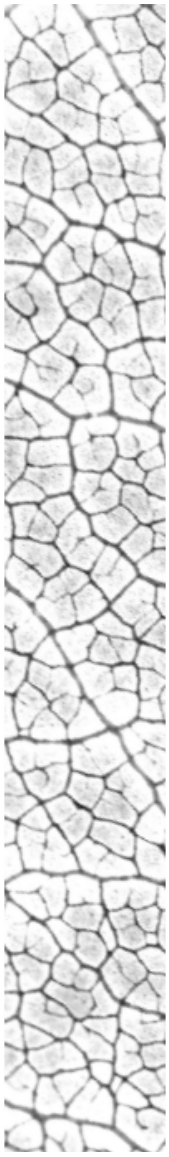

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Isolate rat heart: age features

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Abstract
The autonomic nervous system is involved in the regulation and formation of the heart rhythm. Most experimental studies about the influence of the autonomic nervous system on heart activity in ontogenesis were carried out on a whole organism or isolated tissue. An isolated heart by Langendorff can study arrhythmia, coronary vascular function, and can also help to understand the pathophysiology of modern human diseases, such as hypertension, diabetes, heart failure, or ischemia / reperfusion injury. The work is devoted to the study of isolated heart operation parameters according to Langendorff in postnatal ontogenesis with different levels of adrenergic innervation development. The analysis of the results showed that the stabilization of the isolated heart rate located in a perfused solution occurs during the 16th minute on average. The minimum heart rate values were recorded among newborn animals, the maximum heart rate was detected among 21 day old rats. CP stabilization in the studied age groups was recorded at different time intervals. 7 and 21 day old rats demonstrated the stabilization during the 16th minute, and 42 - 100-day old rat demonstrated such stabilization during the 14th minute. The minimum CP values were recorded among 7 and 21 day old rats; the maximum CP indices were found in adult rats. The stabilization of the contraction force among 42 and 100 day old animals was recorded during the 16th minutes. The minimum values of LVP were recorded in adult rats, the maximum values of LVP were found among 42 day old rats.

Keywords: isolated heart, heart rate, coronary flow, sympathetic innervation of heart, rat, postnatal ontogenesis

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INTRODUCTION

The autonomic nervous system (ANS) regulates many body functions. This is a body control system, implemented by the work of parasympathetic and sympathetic fibers. The sympathetic system, combined with its central nuclei and adrenal medulla, is also known as the adrenergic system. The ANS is involved in the regulation and development of the heart rhythm (Zefirov and Svyatova 1997, Ziiatdinova et al. 2003), however, the priority of its department development remains the object of constant research and discussion.

At the stage of the embryonic period, the activity of the heart is regulated by catecholamines circulating in blood (Taylor et al. 2014). Even before the development of the autonomic nervous system, catecholamines regulate the embryo heart (Lehmann et al. 2013).

According to the data obtained by silvering, by the 28th day of intrauterine development, the heart of a human embryo begins to contract, and the signs of myocardial innervation are determined only by the 35th day of development. Then, the formation of the spinal cord ganglia occurs from the 56th to the 63rd day. They start to reveal the main mediators of the autonomic nervous system in the heart tissues. Then, at different times, sympathetic and parasympathetic nerve endings are formed since the 3rd month of prenatal ontogenesis. After birth, active maturation of the heart innervating structures is observed. The density of sympathetic and parasympathetic fibers in the heart muscle of a person becomes very high during the first 7-10 years of postnatal ontogenesis. The maximum level of nerve plexuses in the heart muscle is observed at puberty. At the age of 35 - 40, they observe the decrease of adrenergic plexus density in the heart muscle, which characterizes the involution of the heart sympathetic nerve structures. There is the blood pressure increase as a result of adrenergic plexus density decrease in the myocardium and the increase of adrenergic receptor number due to the violation of catecholamine uptake by the sympathetic nerve endings (Shvalev 2012).

The functional adrenergic innervation of the rat heart muscle appears after 3 weeks and forms from the 21st

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to 42nd day of postnatal development, while the cholinergic response is established before birth (Robinson 1996).

Most experimental studies about the influence of the autonomic nervous system departments on the activity of the heart in ontogenesis were carried out on a whole organism or isolated tissue (Zefirov 2011, Zefirov et al. 2007). Chronotropic and inotropic functions of the heart (in vivo) are modulated by the central nervous system, hormonal background and humoral factors. During in vitro studies with the application of individual myocardial tissues, the integrity of the studied system functioning is violated. An ex vivo experimental study on a holistic organ eliminates neurohormonal control and the peripheral vasculature. Thus, according to Langendorff, the study of isolated heart function is determined only by intracardiac properties.

At the end of the 19th century, Oscar Langendorf worked on isolated heart model improvement (Langendorff 1895, Lopukhin 1971). Nowadays, the Langendorff-isolated model of the heart has made an invaluable contribution to understanding the heart physiology. This method allows you to study arrhythmia, coronary vascular function, and can also help to understand the pathophysiology of modern human diseases, such as hypertension, diabetes, heart failure or ischemia / reperfusion injury. In the modern age of science, along with actively developing methods of gene manipulation, protein expression, an isolated heart technique remains one of the main in the study of the physiological mechanisms of myocardial cell signaling (Bell et al. 2011).

In relation with the foregoing, our work was devoted to the study of an isolated Langendorff heart operation parameters in postnatal ontogenesis with different levels of adrenergic innervation development.

METHODS

According to the original description by O. Langendorff, the methodology of the experiment remains unchanged to this day. The heart is cannulated in the aorta and the perfusion buffer is passed retrograde down the aorta, against normal physiological blood flow. The great advantage of this method is that an isolated drug can be viable for several hours according to Langendorff, however, within 1 hour of an isolated heart, the heart rate and contractile activity is decreased by 5-10% (Sutherland and Hearse 2000).

This experimental study was conducted on white laboratory rats, at different stages of postnatal ontogenesis (7, 21, 42, and 100 day old rats - daily animals).

All experiments were carried out in compliance with the ethical standards for the treatment of animals (the recommendations of the local bioethical committee of Kazan Federal University). There are no data in the literature about the effect of urethane on rat cardiovascular system activity (Smirnov 1995), which was administered intraperitoneally at the dose of 800 mg/kg.

The onset of anesthesia was characterized by smooth breathing of the animal, the absence of eyelid movement and vibrissa. Then the chest was opened, the heart was removed and washed in a cold Krebs-Henseleit solution. An isolated heart was perfused on PowerLab 8/35 (ZNI 2019) unit. To register myocardial contractile activity, a latex balloon connected to an ML T844 pressure sensor (ADinstruments, Australia) was placed in the cavity of the left ventricle. They treated systolic pressure, final diastolic pressure and heart rate (HR). The pressure was calculated by the left ventricle (mm of merc. col.) was calculated from the curve, as the difference between systolic and end-dystolic pressure. They also calculated heart rate (bpm), coronary flow (ml/min) among 42 and 100 day old rats. Heart rate was calculated by electrogram among 7 and 21 day old rats. The significance of differences in the studied parameters of an isolated heart was calculated using paired Student's t test. Quantitative results of the study are presented in the form of mean value (M) ± standard error (m). The differences were considered statistically significant at: * - $P < 0.05$, ** - $P < 0.01$, *** - $P < 0.001$.

RESULTS AND DISCUSSION

After the start of perfusion of an isolated rat heart among 7, 21, 42, and 100 day old rats, they observed the decrease of heart rate.

The initial HR values among 100 day old animals amounted to 197.3 ± 15.8 beats/min and during the first 5 minutes they were significantly decreased to 175.6 ± 12.5 beats/min ($p < 0.01$). By the 10th minute of perfusion, the heart rate was 169.1 ± 11 beats/min ($p < 0.01$). Since the 13th minute the stabilization of heart rate was observed and the subsequent change was not observed. The heart rate was 167.6 ± 10.9 beats/min ($p < 0.01$) (**Fig. 1**). The total decrease of heart rate from the initial value until stabilization was 16%.

The initial heart rate was 227.3 ± 23 beats/min among 42-day-old rats. By the 5th minute of perfusion, heart rate decreased to 209.5 ± 20.7 beats/min ($p < 0.001$). By the 10th minute of observation, heart rate continued to decrease to 187 ± 19.2 beats/min ($p < 0.001$). Heart rate stabilization was fixed and did not change since the 15th minute and amounted to 174.3 ± 17.9 beats/min ($p < 0.001$) (**Fig. 1**) and made 24% of the initial value.

Among 21 day old rats, the initial heart rate was 258.6 ± 9.4 beats/min. By the 5th minute, a significant decrease of heart rate was observed to 226.8 ± 10.2 beats/min ($p < 0.001$). Over the next 10 minutes, there was the heart rate decrease to 206.1 ± 12.3 beats/min ($p < 0.001$). Heart rate stabilization in this age group was

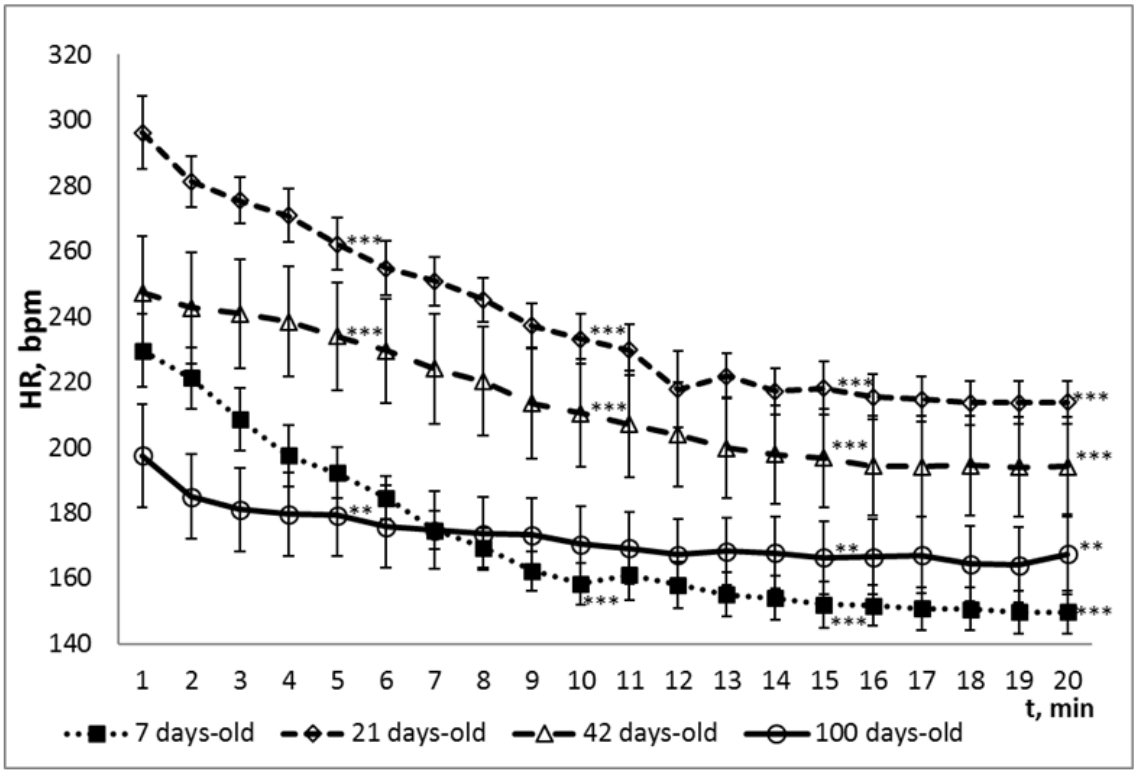

Fig. 1. The dynamics of heart rate indicators for perfusion of rat heart isolated by Langendorff in postnatal ontogenesis. Note: the differences are significant when compared with the initial values: ** - p < 0.01; *** - p < 0.001

recorded during the 17th minute of perfusion. The value was 191.6 ± 13.4 bpm (p < 0.001) (**Fig. 2**). The total decrease of heart rate made 26% from the start of perfusion.

In the age group of 7 day old animals, the initial heart rate was recorded at 229.5 ± 11.1 beats/min. 5 minutes after the start of an isolated heart perfusion, heart rate was 184.5 ± 6.6 beats/min (p < 0.001). By the 10th minute of observation, the heart rate decreased to 160.9 ± 7.6 beats/min (p < 0.001). Heart rate stabilization was observed by the 16th minute of the experiment. Heart rate values were 150.6 ± 6.4 bpm (p < 0.001) (**Fig. 1**) and did not change.

The analysis of experiment series results revealed that when perfusion begins, heart rate has maximum values and decreases over time, after which the stabilization of heart rate occurs by the 16th minute of perfusion on average. The maximum decrease is observed in the age group of 21 day animals and makes 26% (p < 0.001), the minimum decrease of heart rate among 100 day old animals is 16% (p < 0.01). At the final minute of an isolated heart perfusion, the mean heart rate of rats was significantly different in the groups of 7 and 21 day old rats (p <0.01), among 7 and 42 day old animals (p < 0.01) and among 21 and 100 day old rats (p < 0.01) (**Fig. 2**).

The CP of an isolated heart of 100 day-old animals at the 1st minute of observation was 6 ± 0.6 ml/min. By the 5th minute, the CP decreased to 5.89 ± 0.7 ml/min. Then, a gradual decrease of the blood supply to the heart was also observed, and by the 10th minutes it amounted to 5.66 ± 0.7 ml/min (p < 0.01). By the 18th minute of cardiac perfusion, CP stabilized and amounted to 5.47 ± 0.7 ml/min (p < 0.01) (**Fig. 3**). The total change of CP was 10%.

In the age group of 42 day-old rats, the initial value of CP was 5.93 ± 1 ml/min. By the 5th minute of perfusion, the CP decreased to 5.65 ± 1 ml/min (p < 0.05). CP stabilization of an isolated heart was observed by the 13th minute and amounted to 5.24 ± 1 ml/min (p < 0.01) (**Fig. 3**). CP decreased by 12%.

In the group of 21 day old rats, the average CP value was 7.27 ± 0.1 ml/min. By the 5th minute of perfusion, CP began to decrease sharply to 5.54 ± 0.3 ml/min (p < 0.001), by the 12th minute - to 2.37 ± 0.2 ml/min (p < 0.001). Then, the decrease of CP was insignificant; by the 15th minute, CP was 1.52 ± 0.1 ml/min (p < 0.001), and CP stabilization was observed SINCE the 17th minute of perfusion and amounted to 1.35 ± 0.1 (p < 0.001) (**Fig. 3**). The overall decrease in CP was 82%.

In the group of newborn rat, CP was 5.77 ± 0.6 ml/min. By the 5th minute of observation, the CP decreased to 4.11 ± 0.5 ml/min (p < 0.001) and continued to decrease to 2.85 ± 0.3 ml/min (p < 0.001) (**Fig. 3**) during the 15th minute with which the stabilization of the CP began. The overall decline made 51%.

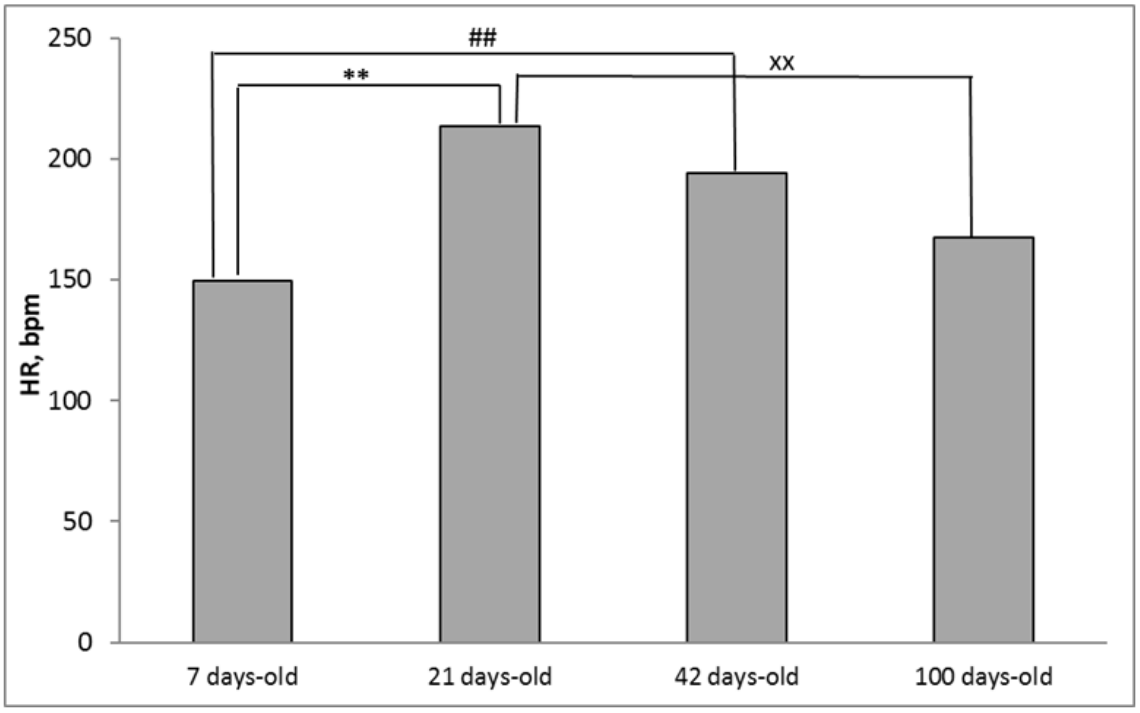

Fig. 2. The change of heart rate in postnatal ontogenesis.
Note: the differences are significant during:
- the comparison of heart rate among 7 day-old and 21 day-old rats: ** - $p < 0.01$;
- the comparison of heart rate among 7 day-old and 42 day-old rats: ## - $p < 0.01$;
- the comparison of heart rate among 21-day-old and 100-day-old rats: xx - $p < 0.01$.

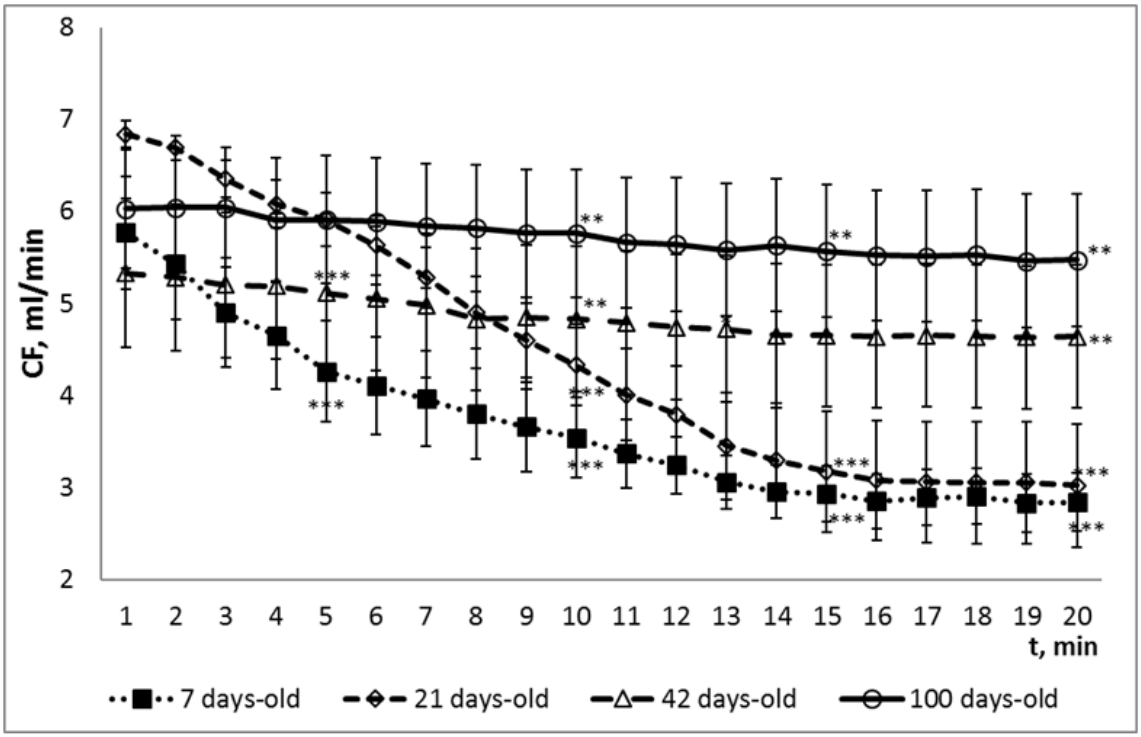

Fig. 3. The change of CP for rat heart perfusion isolated by Langendorff in postnatal ontogenesis. Note: the differences are significant when compared with the initial values: ** - $p < 0.01$; *** - $p < 0.001$.

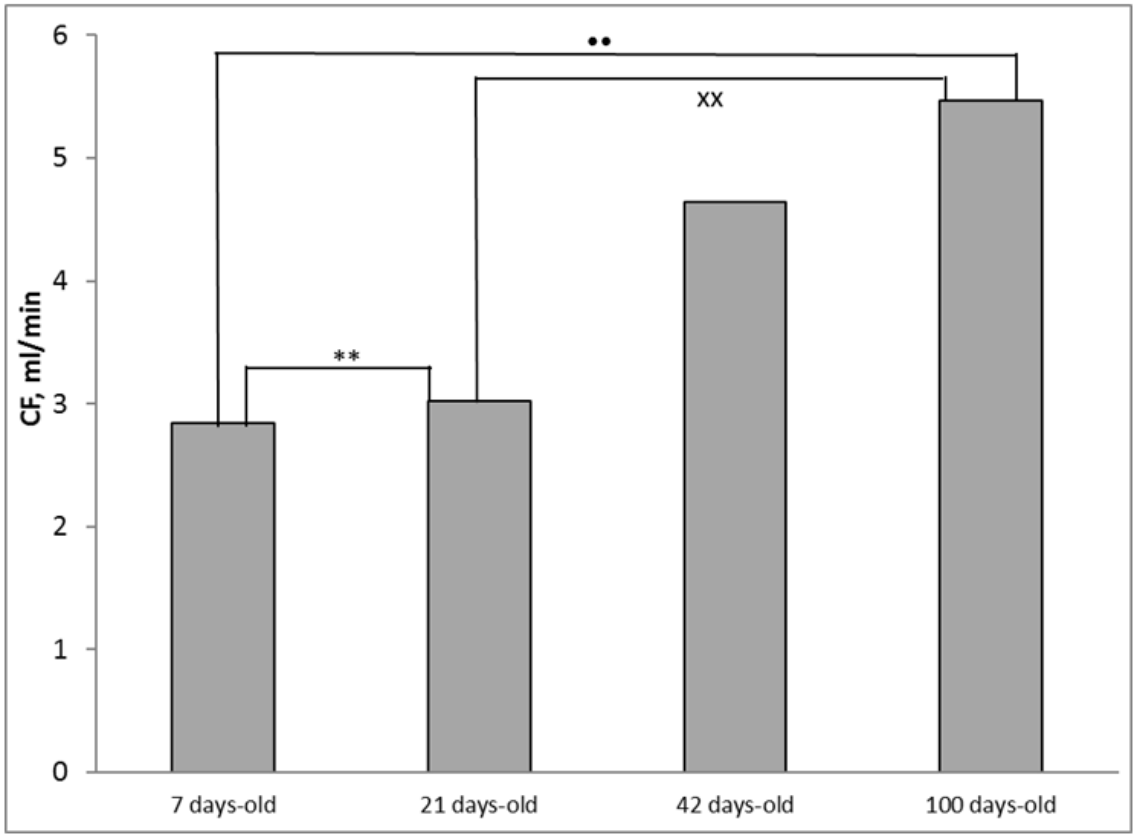

Fig. 4. Change in heart rate during postnatal ontogenesis.
Note: the differences are significant during:
- the comparison of heart rate among 7 day-old and 21 day-old rats: ** - $p < 0.01$;
- the comparison of heart rate among 7 day-old and 100 day-old rats: •• - $p < 0.01$;
- the comparison of heart rate among 21-day-old and 100-day-old rats: xx - $p < 0.01$.

The study of CP among the rats of different ages revealed that this parameter also tends to decrease during the perfusion with the working solution. The maximum decrease in blood supply was observed among 21-day-old animals and made 82% ($p < 0.05$). The minimum decrease CP by 10% was observed among adults ($p < 0.05$). CP stabilization occurred by the 15 minute of an isolated heart perfusion on average. Statistically significant differences in CP changes within the studied age groups were observed by the last minute of observation among the age groups of 7 and 42 day-old rats ($p < 0.01$), 7 and 100 day-old rats ($p < 0.01$), 21 and 100 day-old rats ($p < 0.05$) (**Fig. 4**).

The mean value of LVP among 100 day old rats was 24.9 ± 4.9 mm of merc. col. Then, by the 5th minute of observation, the LVP increased significantly and amounted to 34.3 ± 5.1 mm of merc. col. ($p < 0.001$). By the 10th minute, the LVP increased to 42.6 ± 6 mm of merc. col. ($p < 0.01$). Then, starting from the 13th minute, the LVP stabilized to 44.9 ± 5.5 mm. of merc. col. ($p < 0.01$) (**Fig. 5**) and was maintained at this level.

In the group of 42-day-old animals, the initial value of LVP was 47.4 ± 7 mm. of merc. col. Further, LVP continued to increase to 55.7 ± 7.3 mm of merc. col. ($p < 0.01$) by the 8th minute, up to 60.7 ± 6.9 mm of merc. col. ($p < 0.01$) by the 13th minute. Then, stabilization of LVP was observed, which amounted to 61.5 ± 6.5 mm of merc. col. ($p < 0.01$) (**Fig. 5**). The overall increase in LVP was 30% of the baseline.

Since the first minutes of perfusion, the LVP increased and stabilized by the 16th minutes of observation on average. The maximum increase in LVP is observed among 100 day-old animals by 80% ($p < 0.05$). The comparison of contraction force parameters in these age groups revealed a statistically significant difference only during the first four minutes of an isolated heart perfusion.

SUMMARY

In all age groups, the rate stabilization of an isolated heart located in a perfused solution occurs by the 16th minute on average. The minimum heart rate values were recorded among newborn animals, the maximum heart rate was detected among 21 day-old rats. Stabilization of coronary circulation in the studied age groups was recorded at different time intervals. Among 7 and 21 day-old rats it took place by the 16th minute, and by the 14th minute among 42 and 100 day-old rats. The minimum CP values were recorded among 7 and 21 day-old rats;

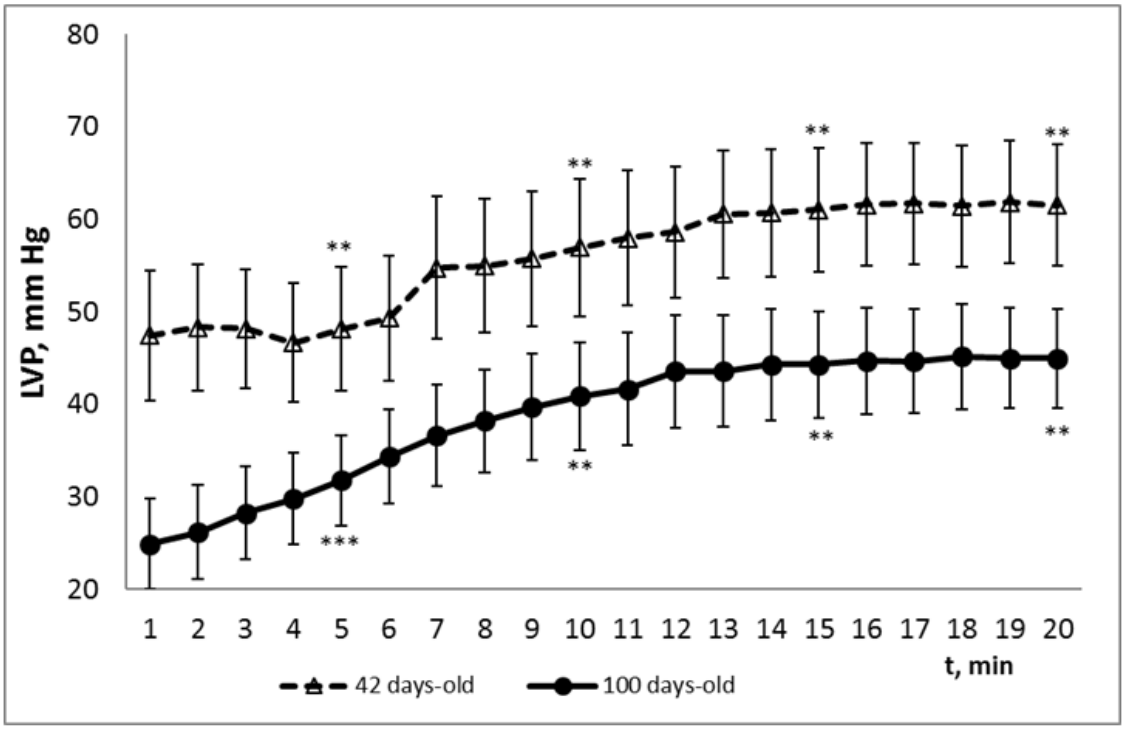

Fig. 5. The change of LVP to the rat heart perfusion isolated by Langendorff in postnatal ontogenesis. Note: the differences are significant when compared with the initial values: ** - $p < 0.01$; *** - $p < 0.001$

the maximum CP indices were found in adult rats. The stabilization of the contraction force among 42 and 100 day-old animals was recorded by the 16th minute. The minimum values of LVP were recorded among adult rats, the maximum values of LVP were found among 42 day old rats.

CONCLUSIONS

Most studies about the influence of the autonomic nervous system departments on the heart activity in ontogenesis were carried out using in vivo and in vitro methods, in which the heart is either controlled by the central nervous and humoral regulation, or has an impaired integrity of the studied system operation. Applying the method of an isolated heart, we were able to exclude the neurohumoral effect on the heart, which may allow us to interpret the physiological mechanisms of the organ more correctly. Our study is devoted to the study of the main indicators of an isolated heart operation at different stages of postnatal ontogenesis with different levels of adrenergic innervation. We recorded the maximum heart rate among 21 day old rats associated with the onset of animal heart sympathetic innervation. Minimum heart rate values were obtained in the age group of 7 day-old rats; the minimum heart rate was associated with the absence of sympathetic innervation of the animal heart. Besides, the data obtained by the Langendorff model of an isolated heart are consistent with the results obtained previously during in vivo experiments (Zefirovet al. 1997).

The study of coronary flow among the rats of different ages revealed that this parameter has the minimum value among 7 and 21 day-old rats, which is associated primarily with the anatomical size of the heart and the lumen of the coronary vessels, as well as with the tone of smooth muscle cells.

The maximum values of pressure developed by the left ventricle were observed among 42 day-old animals. Perhaps a high rate of LVP at this age is associated with the end of the heart sympathetic innervation development, as well as with the beginning of pubertal development of the body.

Thus, the studied parameters of rat heart operation isolated according to Langendorff in postnatal ontogenesis with different levels of adrenergic innervation development are comparable with the results obtained for the whole organism.

CONFLICT OF INTEREST

The author confirms that the presented data do not contain a conflict of interest.

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