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Influence of acute prenatal hypoxia on secretory activity of rat atrial cardiomyocytes during the stages of ontogenesis

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Abstract
We have performed a quantitative ultrastructural analysis of secretory apparatus of rat atrial cardiomyocytes during the stages of ontogenesis after the influence of acute intrauterine hypoxia. Analysis showed that on the 16th day of embryogenesis (3rd day after influence of acute hypoxia) numerical density and relative volume of the secretory granules of rat atrial cardiomyocytes were lower than control group. Experimental group animals had granules of type III in the cytoplasm. Thus, acute hypoxia stimulates the release of atrial natriuretic peptide. However, these changes were transient and were tending to the values the control group on the 18th day of embryogenesis (5th day after the influence of acute hypoxia).

Keywords: *rats, atrial cardiomyocytes, secretory granules, prenatal hypoxia, cardiogenesis.*

1. Introduction
Study of the influence of intrauterine hypoxia on the fetal development is extremely actual problem. Complications from hypoxia are among the top 10 causes of fetal death and elevated risk of adult cardiovascular disease [7]. Atrial cardiomyocytes take part in maintenance of circulating blood volume due to their secretory activity. Because of release of atrial natriuretic peptide (ANP) from cardiomyocytes induced by myocardial stress (atrial distension, tachycardia or acute hypoxia) the plasma level of this peptide is used as diagnostic marker in the clinical setting to monitor the severity of hypertrophy and congestive heart failure [8]. Previous studies were focused on physiological effects of hypoxia on secretory activity of the heart cells [4] whereas morphological changes of cardiomyocytes that underlie this process are still unknown. The purpose of this study was to analyze quantitative and ultrastructural changes of secretory apparatus of rat atrial cardiomyocytes after the influence of acute prenatal hypoxia during the stages of ontogenesis.

2. Materials and Methods
As the material we used rat`s embryo hearts on the 14th, 16th and 18th embryonic day, hearts of newborn rats and hearts of the rats on the 3rd, 14th and 30th day of postnatal ontogenesis. Experiments were carried out in according to the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 2005) and "General ethical principles of animal experiments", adopted by the Fifth National Congress on Bioethics (Kyiv, 2013). Induction of hypoxia was made by standard method [3]. Pregnant females were injected by 1% sodium nitrite solution subcutaneous in dose of 6 mg per 100 g of weight on the 13th embryonic day for modeling of acute hypoxia. Control animals were injected by 1 ml of 0.9% physiological solution of sodium chloride subcutaneously. Fetal hearts were fixed at 2.5% glutaraldehyde solution, postfixed in 1% OsO4 («SPI», USA) and embedded in Epon-812 according to standard protocol. The study was conducted using a transmission electron microscopy. We carried out biometrical [1] and statistical analysis [6].

3. Results and Discussion
Electronmicroscopic analysis has showed that secretory granules of rats embryos atrial cardiomyocytes on 16th embryonic day (3rd day after exposure to acute hypoxia) differed by the number and morphology compared with the control group.
Secretory cells of experimental group animals contained granules of I and III type. Whereas there were only newly formed granules on the cytoplasm of rats control group cardiomyocytes.

It is known that quantitative ratio of secretory granules of various types and ratio of the relative volumes of the Golgi complex and secretory granules in cardiomyocytes shows changes of the heart endocrine activity [5]. Diameter of the granule type I at experimental group animals was reduced by 50.4% ($p<0.05$) compared to the control group (table 1). Lack of type II granules and smaller diameter of type I granules compared with the norm is due to the rapid transition I to type III granules. The numerical density of granules and relative volume of granules in cardiomyocytes of experimental group animals were lower on 50.0% ($p<0.05$) compared with the control group. We suggested that the acute hypoxia stimulated the excretion of atrial natriuretic peptide (ANP). The transcription of natriuretic peptides in hypoxia is mediated by hypoxia-inducible factors (HIFs) which transcriptionally regulate the expression of hundreds of hypoxia-dependent genes in all mammalian cell types [2]. Stimulus for the synthesis and release of natriuretic peptides is the oxygen gradient which always occurs in all human tissues in physiological conditions. The plasma volume decrease caused by natriuretic peptides (natriuresis, diuresis, and plasma shift) leads to hemoconcentration and ultimately to the increased oxygen-carrying capacity of unit volume of blood. Thus, acute hypoxia stimulates more active release of ANP as a compensatory response to oxygen deficiency.

Table 1: Morphometric parameters of secretory activity of rat atrial cardiomyocytes during ontogenesis, M ± m

The period of development	Age, day	Research group	Numerical density of granules, 1/100 nm²	Diameter of granules, nm			The relative volumes of secretory structures, %		The quantitative ratio of granules various types, %		
				I	II	III	Golgi complex	Secretory granules	I	II	III
Embryonic	14	Experimental	–	–	–	–	2.5±0.3	–	–	–	–
		Control	–	–	–	–	3.1±0.4	–	–	–	–
	16	Experimental	4.53±0.52*	79.4± 8.1*	–	82.2± 8.3*	2.1±0.3	0.3±0.1*	75.0± 7.8	–	25.0± 2.6*
		Control	8.3±0.9	160.2 ±17.3	–	–	2.3±0.3	0.5±0.1	100	–	–
	18	Experimental	30.5±3.5	169.5 ±17.8	180.1 ±17.9	155.5 ±13.8	2.2±0.3	1.8±0.2	12.2± 1.5	48.2± 5.2	39.6± 4.1
		Control	34.9±3.6	180,2 ±20,3	185,2 ±20,5	165.3 ±16.9	2.3±0.3	2.0±0.2	12.3± 1.5	52.4± 5.5	35.3± 3.5
Postnatal	1	Experimental	25.3±2.6	189.2 ±20.1	200.5 ±18.3	160.0 ±16.3	2.5±0.4	1.8±0.2	7.0± 0.8	38.0± 2.8	55.0± 5.6
		Control	26.2±2.8	200.6 ±21.6	210.0 ±23.3	160,8 ±17.3	2.1±0.2	1.5±0.2	8.2± 0.9	38.5± 4.1	53,3± 5,7
	3	Experimental	10.2±0.2	235.1 ±23.6	240,1 ±23.3	180.0 ±20.5	2.3±0.3	1.2±0.1	13.8± 1.4	18.2± 1.9	68.0± 6.8
		Control	13.6±1.4	230.5 ±24.8	242.8 ±26.2	200.6 ±23.5	2.2±0.3	1.0±0.1	14.8± 1.5	22.2± 2.4	63.0± 6.6
	14	Experimental	8.0±0.8	235.0 ±23.6	241.5 ±25.7	200.3 ±22.9	2.0±0.3	1.1±0.2	28.5± 2.9	19.4± 2.1	52.1± 5.8
		Control	10,4±1,1	240.6 ±24.9	245.0 ±25.8	203.4 ±22.8	1.8±0.2	1.0±0.1	30.3± 3.3	19.7± 2.2	50.0± 5.6
	30	Experimental	29.2±3.7	238.1 ±23.8	245.4 ±25.3	210.5 ±25.1	1.6±0.2	1.6±0.2	–	25.5± 2.6	74.5± 7.8
		Control	30.0±2.8	240.8 ±24.9	252.3 ±26.4	213.3 ±22.2	1.5±0.1	1.5±0.2	–	24.2± 2.6	75.8± 7.7

Note: * – $p< 0.05$ – significance of differences with the control group.

On the 18th embryonic day in atrial cardiomyocytes of experimental and control groups animals there were three types of secretory granules that were morphologically differed: type I – newly formed granules, type II – mature granules and type III – diffused granules. On the 5th day after acute hypoxia exposure rat atrial cardiomyocytes were not different comparing with the normal secretory activity. Secretory granules were mainly united in groups, more rare they were arranged solitary. Number of the granules in cardiomyocytes was predominant in the right atrium. At the same time content of the granules in atriums were less than in auricles. Numerical density of the granules and relative volume of the granules in experimental group animals were increased in 6.3 ($p<0.05$) and 7.2 ($p<0.05$) times compared with the previous term but were not significantly different from the control group. In both groups diameter of the various types of granules, the relative volumes of Golgi complex and secretory granules were not significantly different. Number of the granules type II was predominant (48.2±5.2%) in secretory cells of experimental group animals. Number of the granules type I and III was 39.6 ±4.1 and 12.2%±1.5%, respectively and was not significantly different from the norm. It is indicates active accumulation of the granules in this period.

On the 1st day of postnatal development of atrial cardiomyocytes of experimental group animals did not differ in the number and profile of the granules from the control group. Numerical density of the granules decreased compared with the previous term in both groups. In the cytoplasm of the

secretory cells of the experimental group animals type III granules were represented the highest percentage – 55.0±5.6%. Number of the granules type I was 7.0±0.8% and type II – 38.0 ±2.8%. That was not different significantly from the control group and indicates rapid release of the granules from atrial cardiomyocytes and associated with changes in hemodynamic conditions on this term. Start of the pulmonary circulation after birth leads to a sharp increase of the blood pressure in the left atrium and mechanical stretching of the atrial wall. As it well known [4], it is a direct and immediate stimulus for the release of ANP.

On the 3rd day of postnatal development numerical density of granules and relative volume of the granules in atrial cardiomyocytes of experimental group animals decreased in 2.5 ($p<0.05$) and 1.5 times ($p<0.05$), respectively compared to the previous term but were not significantly different from the control group. Rat atrial cardiomyocytes of both groups were characterized by prevalence of type III granules (fig. 1). That indicates rapid release of ANP on this term and confirmed by previous studies [5].

On the 14th day of postnatal development number of granules of type III was 52.1±5.6% and I type was 28.5±3.3% in atrial cardiomyocytes experimental group animals. Granules type II were the lowest percentage (19.4±2.2%). The relative volume of the Golgi complex was two times ($p<0.05$) higher than the volume of secretory granules on this term. This indicates intensification of the processes of granules formation. Thus on this term the synthesis and rapid release of ANP prevailed over the processes of accumulation.

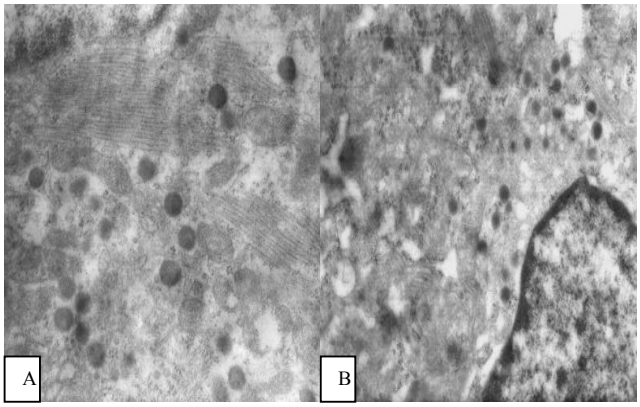

Fig 1: Secretory apparatus of rat atrial cardiomyocytes on the 3rd day of postnatal development of experimental group (A) and control group (B). Electron micrographs. A – ×20000, B – ×15000.

On the 30th day of postnatal development numerical density of granules increased in 3.8 times ($p<0.05$) and the relative volume increased on 60.0% ($p<0.05$) in experimental animals atrial cardiomyocytes compared with the previous term. Profile of the granules was also changed compared with the previous period. First place by the number of granules was taken by type III, second – by II type and granules of type I were not observed at all. This showed rapid transition of the granules type I into II and III type and was due to the intensive release of ANP from atrial cardiomyocytes. Total increase of granules number was due to the formation of urinary and reproductive systems and development of renin-angiotensin-aldosterone system. That is consistent with previous studies [5]. Rapid release of the granules was associated with increased demand of ANP diuretic effect due to the constantly increasing hemodynamic burden on the atrial myocardium on this term.

Thus, there was more active release of ANP on the 3rd day after exposure of acute hypoxia, which was compensatory body's response to oxygen deficiency. On the following study terms (from the 5th day after exposure of acute hypoxia) secretory activity of atrial cardiomyocytes of the experimental group of animals was gradually approached to the norma, and was indicated transient changes.

4. Conclusion

Rats atrial cardiomyocytes on the third day after the influence of acute hypoxia have lower numerical density and relative volume of granules than normal values. Granules of type III are present. This indicates acute hypoxia stimulation of atrial natriuretic peptide release. Changes that were revealed returned to normal on the fifth day after influence acute hypoxia.

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