Tissue Homeostasis of the Myocardium in Newborn Albino Rats Exposed to Intrauterine Hypoxia

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Somatometric indexes were modified in the offspring of rats exposed to hypobaric hypoxia. DNA synthesis and tissue mitotic index increased in 5-day-old male rats, but underwent less pronounced changes in females. Our results indicate that hypoxia modulates morphogenesis of the myocardium. We revealed sex differences in the reaction of newborn albino rats to intrauterine hypoxia.

Key Words: *intrauterine hypoxia; myocardium; proliferative activity*

Intrauterine hypoxia (IUH) is the major mechanism underlying damage to the mother-fetus system [1]. IUH constitutes 21-45% perinatal morbidity rate [9]. The incidence of posthypoxic circulatory disturbances in newborns reaches 40-70% [8]. However, little is known about the influence of IUH on postnatal morphogenesis of the myocardium [7,13]. Here we studied indexes of structural homeostasis in the myocardium of newborn albino rats exposed to IUH.

MATERIALS AND METHODS

Experiments were performed on female rats aging 5-6 months and weighing 200-220 g. Hypoxia was modeled on days 14-19 of pregnancy. The animals were maintained in an altitude chamber for 4 h (height 9000 m, Po_2 42 mm Hg). Rat pups were decapitated on day 5 after birth. The control group included 5-day-old rat pups from intact females. The study was conducted on 173 animals.

DNA synthesis was determined by ^{3}H-thymidine autoradiography. Newborn rats intraperitoneally received ^{3}H-thymidine in a dose of 1 μCi/g (specific activity 84 TBq/mol) 1 h before euthanasia. Autoradiographs were prepared as described elsewhere [5]. The index of labeled nuclei (ILN) was estimating by counting 1000 cells in subendocardial layers of the myocardium in each region of the heart (ventricles, interventricular septum, and atria). The labeling intensity (LI) was calculated as the mean number of tracks per 50 nuclei in each myocardial region. Mitotic activity was determined on preparations stained with hematoxylin and eosin. We assayed not less than 5000 nuclei in each myocardial region. The mean number of nucleolar organizer regions in the nuclei was evaluated on preparations stained with $AgNO_3$ [6]. To evaluate sex differences in the reaction to IUH, these indexes were studied in males and females.

Intergroup differences were significant at $p<0.05$.

RESULTS

Somatometric indexes were changed in rat pups exposed to IUH. Body weight in these rats decreased by 8.76% compared to control animals (7.31±0.21 and 7.95±0.14%, respectively). It should be emphasized that the relative weight of the heart in animals exposed to IUH increased by 7.3% compared to the control (7.32±0.11 and 6.82±0.11, respectively). Previous studies showed that IUH is followed by a decrease in body weight [11,15] and increase in the relative weight of the heart [15].

ILN increased, while LI remained practically unchanged in various myocardial zones of male rats exposed to IUH (Table 1).

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TABLE 1. Effect of IUH on DNA Synthesis in the Myocardium of Newborn Albino Rats (*M±m*)

Heart region	Control		IUH	
	ILN, %	LI	ILN, %	LI
Male rats				
Left atrium	8.00±0.39	27.12±1.76	10.05±0.55*	28.76±1.76
Left ventricle	8.81±0.79	27.56±1.46	10.51±0.62*	27.89±1.10
Interventricular septum	8.68±0.65	30.15±1.69	10.80±0.73*	28.83±1.38
Right atrium	7.24±0.42	28.34±1.88	9.69±0.43*	27.44±1.23
Right ventricle	7.21±0.45	26.08±1.25	9.38±0.44*	28.75±1.42
Female rats				
Left atrium	8.83±0.58	26.36±1.01	8.43±0.60	26.99±0.43
Left ventricle	8.57±0.40	28.14±0.55	11.00±0.55*	25.81±0.88*
Interventricular septum	8.55±0.59	27.99±0.54	9.64±0.34	25.65±1.08
Right atrium	8.03±0.53	23.88±1.23	8.27±0.91	25.58±0.92
Right ventricle	7.73±0.45	26.68±0.63	8.26±0.43	26.59±1.12

Note. Here and in Table 2: *$p<0.05$ compared to the control.

The number of mitotic figures in the myocardium of male rats significantly increased after IUH (by 92.5%, Fig. 1).

Our results indicate that IUH increases proliferative activity of the myocardium in rat pups, which is consistent with published data [13]. This level of mitotic activity is typical of cardiomyocytes at earlier stages of ontogeny [7]. Therefore, the effect of IUH can be related to retarded development and immaturity of the myocardium in experimental animals [11,15]. Previous studies on cultured cardiomyocytes from newborn rats showed that hypoxia induces gene expression, which is usually observed at the early stage of cardiogenesis [14].

Published data show that hypoxia activates the synthesis of endothelial growth factor in cardiomyocytes, which is followed by auto- and paracrine growth stimulation [10]. The number of nucleolar organizer regions tended to increase in different myocardial regions of treated male rats, which reflects activation of anabolic processes in the myocardium (Table 2).

The increase in proliferative activity of cells can serve as a compensatory response to high-intensity apoptosis in cardiomyocytes produced by hypoxic stress [12]. Our previous studies showed that activation of DNA synthesis in epithelial tissues of adult animals exposed to hypoxia has a compensatory role [2].

Hypercatecholaminemia probably plays a major role in the pathogenesis of changes observed in newborn animals after chronic IUH [8]. Catecholamines affect proliferative activity of tissues in experimental animals during the early postnatal ontogeny, which

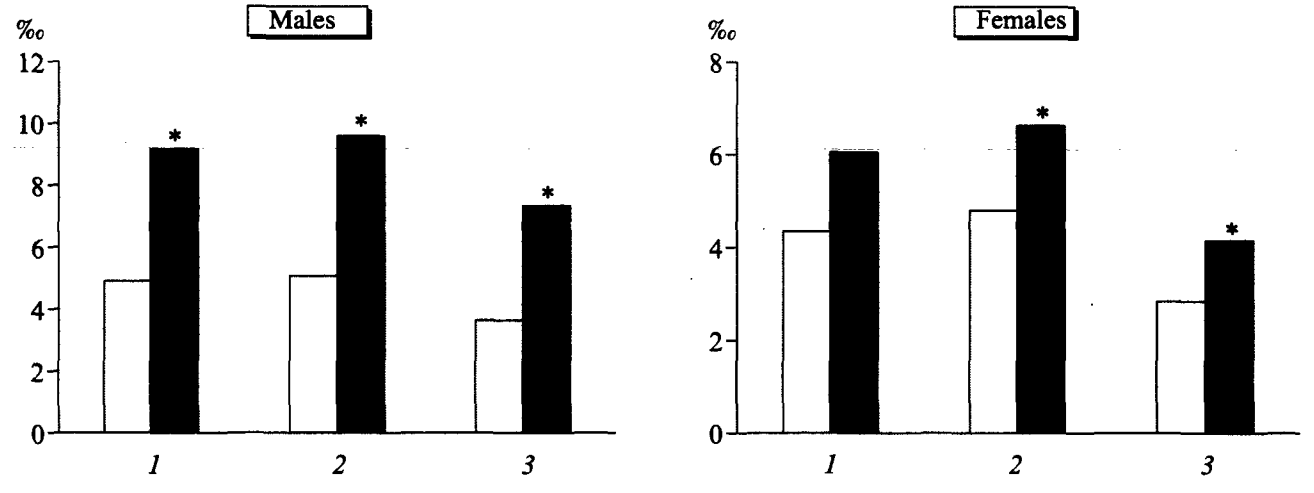

Fig. 1. Effect of intrauterine hypoxia on mitotic index of the myocardium in newborn rats: left ventricle (*1*), interventricular septum (*2*), and right ventricle (*3*). Light bars: control. Dark bars: treatment. *$p<0.05$ compared to the control.

TABLE 2. Effect of IUH on the Number of Nucleolar Organizer Regions in Cardiomyocyte Nuclei of Newborn Albino Rats (*M±m*)

Heart region	Number of nucleolar organizer regions	
	control	IUH
Male rats		
Left ventricle	3.097±0.184	3.389±0.163
Interventricular septum	3.057±0.177	3.454±0.147
Right ventricle	2.966±0.135	3.397±0.203
Female rats		
Left ventricle	3.386±0.083	3.581±0.122
Interventricular septum	3.520±0.079	3.527±0.148
Right ventricle	3.257±0.185	3.769±0.114*

manifested in deceleration of mitotic processes. The increase in catecholamine concentration in newborn animals is followed by mitosis arrest in metaphase [3]. We observed a significant decrease in the prophase-metaphase index of the ventricular myocardium in male rats of the IUH group (by 2.0-2.6 times).

As differentiated from newborn rats exposed to IUH, sex differences were not found in control animals. In newborn female rats exposed to IUH autoradiography revealed pronounced changes in DNA synthesis only in the subendocardial layer of the left ventricle (Table 1). The increase in ILN in this myocardial region was accompanied by a significant decrease in LI. These changes reflected deceleration of DNA synthesis and artifactual changes in ILN. They were associated with an increase in the count of S-phase cells. The mitotic index of the myocardium in IUH females increased less significantly than in males (Fig. 1). In female rats, pronounced changes were observed only in the myocardium of the interventricular septum and right ventricle. No shifts were revealed in phases of mitosis. The nucleolar apparatus of right ventricular cardiomyocytes was activated in females of the IUH group (by 13.6%, Table 2).

Our results suggest that posthypoxic structural changes in the myocardium of newborn female rats were less pronounced than in males. Minor differences in proliferative activity were found in the myocardium in newborn treated females and control animals. The study of the nucleolar organizer regions revealed intensive anabolic processes in the myocardium of female rats exposed to IUH. These data illustrate great compensatory reserves of female rats in the early period of ontogeny. Male fetuses were more sensitive to IUH. Higher resistance of females to stress was reported previously [4]. For example, the number of 7-day-old females highly resistant to hypoxia 4.7-fold surpasses that of males [1].

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