Effects of Antenatal Hypoxia on Tissue Homeostasis in the Myocardium of Albino Rats: Early and Delayed Consequences

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Body weight, absolute and relative heart weight, DNA-synthetic activity of cardiomyocytes, and number of nucleoli in cardiomyocyte nuclei were reduced in newborn albino rats exposed to antenatal hypoxia. All these changes developed against the background of oxidative stress. In mature animals, the absolute and relative heart weights were decreased, but the ratio of mononuclear cardiomyocytes in both ventricles was higher than in the control. These changes were accompanied by an increase in the percent of collagen fibers in the myocardial stroma.

In the right ventricle, an increase in the cardiomyocyte length to width ratio and a decrease in the number of nucleoli were found. The observed changes can probably induce heart function disturbances under pathological conditions.

Key Words: antenatal hypoxia; cardiomyocytes; oxidative stress

Fetal hypoxia is a universal damaging factor in the mother–fetus system [4]. Antenatal hypoxia induces significant disadaptation of the cardiovascular system of children during perinatal ontogeny [9]. We have previously showed changes in the parameters of tissue homeostasis in the myocardium of 5-day-old albino rats exposed to antenatal hypoxia [6]. Exposures to antenatal hypoxia can play a pivotal role in the development of cardiovascular pathology at the following stages of ontogeny [11]. Antennal hypoxia significantly increases the risk of myocardial function impairment in the experimental model of myocardial ischemia–reperfusion in mature animals [15].

Here we studied early and delayed disturbances of tissue homeostasis in the myocardium of albino rats exposed to antenatal hypoxia.

MATERIALS AND METHODS

Experiments were performed on female outbred albino rats. The animals were kept under standard conditions, standard regimen and ration. The experiments were conducted in accordance to the Order No. 267 of the Ministry of Health of Russian Federation (June 19, 2003) and Provision on Ethical Principles of Animal Experiments of the Far-Eastern State Medical University (February 01, 2012).

Hypoxia was modeled by hypobaric exposure (“height” 9000 m, 4 h). Partial oxygen pressure in an altitude chamber was decreased to 42 mm Hg. Some pregnant female rats were exposed to hypoxia on gestation days 14-19, other females were intact (control). The samples were taken from the offspring (males) on postnatal days 1 (newborns) and 60 (mature rats). The study was conducted on 80 animals, each experimental group consisted of 10-15 animals. Body weight and absolute and relative heart weights (heart index, the ratio between absolute heart and body weights) were measured. Gravimetric measurements were performed...
on an electronic balance after washing of unfixed tissue with physiological saline and careful drying with filter paper.

Free radical oxidation in heart homogenates were estimated by chemiluminescence method (CL) on an LS 50B luminescence spectrometer (PerkinElmer, Inc.). The signal was standardized using an embedded Finlab software. The following parameters of spontaneous and stimulated luminescence were evaluated [1]: Ssp, light sum over 1 min of spontaneous CL, which directly correlates with the intensity of free radical generation; H1, maximal amplitude of fast flash of Fe2+-induced CL reflecting the presence of lipid hydroperoxides; Sind1, light sum over 2 min of Fe2+-induced CL reflecting the rate of peroxide generation; H2, maximal amplitude of H2O2-induced luminol-dependent CL that indirectly correlates with substrate resistance to peroxides; Sind2, light sum over 2 min of H2O2-induced luminol-dependent CL indirectly correlating with activity of antioxidant antiradical defense. CL intensity (mV) was calculated per 1 mg tissue and expressed in rel. units.

Analysis of DNA-synthetic activity of cardiomyocytes (CM) was performed by autoradiography. H3-thymidine in a dose of 1 μCi per 1 g body weight (specific activity 84 Ci/mol) was administrated to animals 1 h before sacrifice. Routinely processed histological sections were mounted on degreased slides, deparaffinized, and covered with nuclear emulsion (Kodak). After 14-day incubation, the films were stained with D-19 solution, fixed with 33% sodium hyposulphite, and stained by hematoxylin and eosin. Nuclear labeling index (NLI) was estimated after examination of 1000 nuclei in CM from subendocardial layer of ventricular myocardium and expressed in percent. The number and ratio of mono- and polynuclear CM were analyzed using the method of alkaline myocardium dissociation [8] followed by staining with hematoxylin and eosin. The CM length to width ratio (LW ratio) was determined by morphocytometry using Mekos-Ts image analyzer. CM nucleolar apparatus was studied on histological sections stained with silver nitrate. The mean number of nucleoli in one CM nuclei was measured after examination of at least 100 nuclei [7]. Quantitative parameters of collagen matrix of myocardial connective tissue (in %) were estimated by morphometry using Avtandilov eyepiece graticule and light microscopy of sections stained by van Gieson’s method. Statistical analysis of data was performed by Statistica 6.0 software.

RESULTS

Antenatal hypoxia induced a significant decrease in body weight of newborn animals by 15.9% (5.61±0.24 vs. 6.67±0.27 g in the control; p=0.005).

The absolute and relative weights of the heart in 1-day-old animals exposed to antenatal hypoxia decreased by 27.2 and 12.9%, respectively, (Table 1).

Analysis of DNA synthesis in the myocardium of left ventricle showed that NLI decreased by 28.9% in the treatment group; the number of nucleoli in CM nuclei of subendocardial layer of left ventricle significantly decreased (by 9.7%).

Structural changes in the myocardium of newborn animals from treatment group were associated with manifestations of oxidative stress at the organ level. Analysis of CL parameters in heart homogenates of newborn animals showed that antenatal hypoxia promoted intensification of free radical oxidation in the tissue (Table 2). Under these conditions, Ssp increased by 1.93 times. An increase in the level of hydroperoxides (H1 amplitude increased by 1.75 times) and accelerated production of peroxyl radicals (Sind1 increased by 1.91 times) indicate that LPO activation significantly contributed in this process. The observed impairments of oxidative state were determined by impaired anti-oxidant defense (Sind2 increased by 2.01 times) and reduced resistance to LPO (H2 amplitude increased by 2.8 times).

Thus, in animals exposed to antenatal hypoxia structural deficiency of the myocardium forming during early neonatal period is accompanied by significant oxidative stress at organ level. Previously we

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group (n=15)</th>
<th>Treatment group (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute heart weight, mg</td>
<td>44.73±2.75</td>
<td>32.55±1.80*</td>
</tr>
<tr>
<td>Relative heart weight, mg/g</td>
<td>6.66±0.19</td>
<td>5.80±0.18*</td>
</tr>
<tr>
<td>NLI of subendocardial layer of myocardium in left ventricle, %</td>
<td>13.92±1.17</td>
<td>9.90±1.05*</td>
</tr>
<tr>
<td>Nucleolus number in the nuclei of CM from left ventricle</td>
<td>2.89±0.06</td>
<td>2.61±0.03*</td>
</tr>
</tbody>
</table>

Note. Here and in Table 2 and 3: *p<0.05 in comparison with the control.