PHYSIOLOGY

Effect of Hypoxia during Early Organogenesis on Cardiac Activity and Noradrenergic Regulation in the Postnatal Period

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Cardiac activity in rats during the postnatal period was studied *in vitro* and *in vivo* after exposure of rat pups to antenatal acute hypobaric hypoxia at the stage of organogenesis (day 9-10 of gestation). Cultured cardiomyocytes from rat pups exposed to antenatal hypoxia were characterized by increased rate of contractions and decreased reactivity to norepinephrine. Heart rate elevation, predominance of sympathetic influences on cardiac activity, and significant increase in norepinephrine concentration in the cerebral cortex were found in freely moving animals exposed to antenatal hypoxia. Our results indicate that hypoxia at the stage of organogenesis modulated cardiac activity during the postnatal period, which manifested at the level of effector structures in the heart and activity of regulatory systems.

Key Words: *antenatal hypoxia; organogenesis; cardiomyocytes; ECG; noradrenergic regulation*

Antenatal hypoxia often leads to severe irreversible complications, which manifest in dysfunction of various systems in the maternal organism and fetus (*e.g.*, cardiovascular system) [5].

Chronic experiments showed that rat pups from females exposed to acute hypoxia at the progestational stage (days 4-5 of pregnancy) are characterized by abnormal development. Hypoxia considerably modulates cardiac activity, including the chronotropic response. These changes develop against the background of autonomic imbalance and significant increase in norepinephrine (NE) concentration in the brainstem of rat pups.

Early organogenesis is another important stage of pregnancy (days 9-10 of pregnancy in rats). Exposure to acute hypobaric hypoxia (AHH) during this period also affects cardiac chronotropic activity in the offspring. These changes are associated with a direct effect of AHH on the development of atrial pacemaker cardiomyocytes (CM) and with indirect neurohumoral regulatory influences.

In the present work, cultured CM from newborn rats exposed to acute hypoxia during early organogenesis were used as a model system to study *in vitro* the chronotropic response and reactivity to NE. It was also interesting to compare these findings

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with ECG parameters of postpubertal rats from the same females and with changes in the concentration of NE in the cerebral cortex.

MATERIALS AND METHODS

Experiments were performed on the offspring of outbred albino rats exposed to AHH on days 9-10 of intrauterine development (experimental group) or intact (control group). AHH was modeled in an altitude chamber at 145 mm Hg, which corresponded to a height of 11,500 m above sea level. The "ascent" took 1 min. AHH session was stopped after short-term cessation of breathing (the exposure lasted 152 sec on average).

The atria were isolated from 2-3-day-old rat pups in series I [2]. CM were obtained after trypsin treatment [3] and cultured on coverslips in penicillin flasks with 2 ml culture medium.

Contractile activity of CM was recorded before and after addition of NE in increasing concentrations (10^{-10}-10^{-7} M) to the culture medium. The measurements were performed using a photometric ocular microscope [4]. Before addition of the next NE concentration baseline chronotropic activity of CM was restored by repeated replacing of the culture medium.

Changes in the contraction rate of CM induced by NE in various concentrations were expressed in percents of baseline chronotropic activity. The most significant effects were observed 1-3 min after replacement of the culture medium for the medium with NE in test concentration. The differences between the absolute values in control and NE-treated rats were evaluated by nonparametric Mann—Whitney *U* test.

In series II we compared ECG parameters of 2-month-old control and experimental rats freely moving in a hole-board chamber. Electrodes for ECG monitoring were subcutaneously implanted to nembutal-narcotized animals (30 mg/kg intraperitoneally) 1 day before the study. ECG was recorded in one of the standard leads over 2 min using ISCOUP software. This procedure allowed us to construct the time curve for an analog signal that was digitized at 500 Hz. The data were processed using Spike-C3 and Intervals 1.02 softwares. We evaluated the mean *R-R* interval, heart rate (HR) variability (scatter of data, ΔX, msec), mode amplitude (AMo, ratio of the most frequently occuring *R-R* interval, %), and monotony index (K_M=AMo/ΔX). The latest 3 parameters allowed us to evaluate the autonomic balance in the regulation of cardiac activity [1].

NE concentration in the cerebral cortex was measured in rats of the same age groups [6].

RESULTS

The study of the chronotropic response showed that cultured atrial CM from rat pups of the experimental group exhibit higher contraction rate compared to control animals (Fig. 1, *a*).

Intergroup differences were revealed in the response to NE. Rats of the experimental group exhibited lower positive chronotropic responses to NE in concentrations of 10^{-7}-10^{-9} M compared to control animals (Fig. 1, *b*).

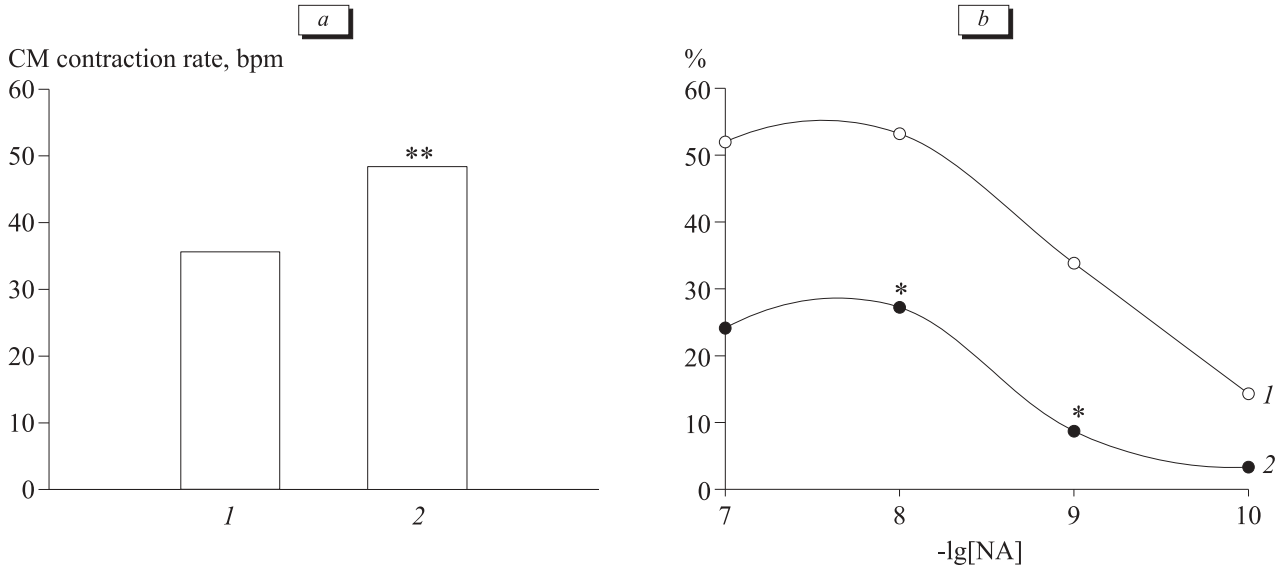

Fig. 1. Effect of acute prenatal hypoxia (*a*) and administration of NE in increasing concentrations (*b*) on contractile activity of CM. Control (*n*=28, *1*) and experimental groups (*n*=29, *2*). *p<0.05 and **p<0.01 compared to the control.

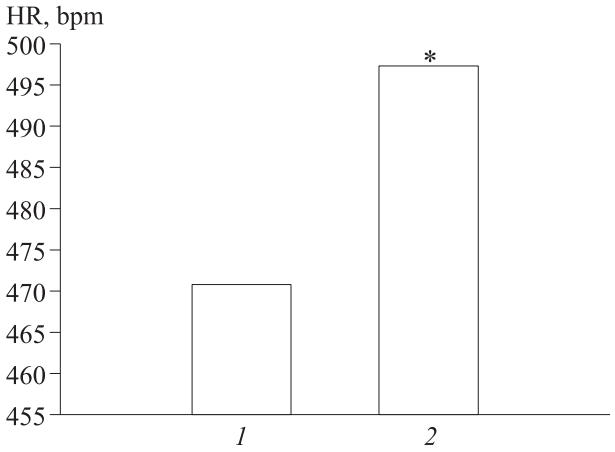

Fig. 2. Effect of acute prenatal hypoxia on HR in adult offspring. Control (*n*=37, *1*) and experimental group (*n*=43, *2*). **p*<0.01 compared to the control.

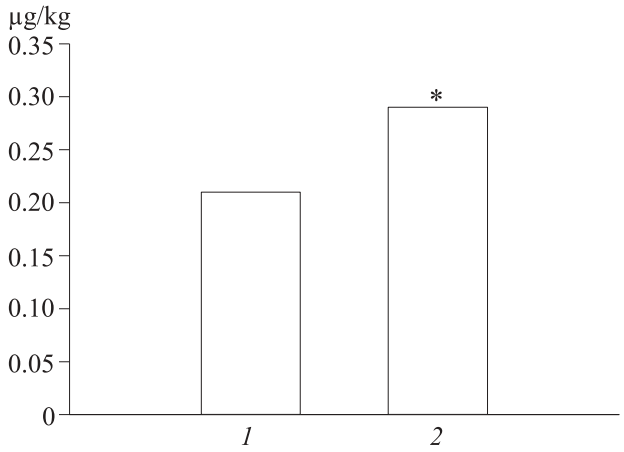

Fig. 3. Effect of acute prenatal hypoxia on NE concentration in the cerebral cortex of adult offspring. Control (*n*=35, *1*) and experimental group (*n*=32, *2*). **p*<0.05 compared to the control.

TABLE 1. Autonomic Balance in 57-60-Day-Old Rats

Group	AMo	K_M	ΔX
Control (*n*=37)	6.5	0.13	70.4
Experimental (*n*=43)	9.8**	0.29**	54.1*

Note. **p*<0.05 and ***p*<0.01 compared to the control.

These data show that exposure to AHH on days 9-10 of gestation has a strong modulatory effect on the chronotropic response and reactivity of atrial CM to NE.

ECG monitoring showed that HR in 57-60-day-old freely moving rats of the experimental group is much higher compared to control animals of similar age (Fig. 2). Significant increase in AMo and K_M was accompanied by a decrease in HR variability (ΔX, Table 1). NE concentration in the cerebral cortex of treated rats was 35% higher compared to control animals (p<0.05, Fig. 3).

Our results indicate that AHH exposure at the stage of organogenesis irreversibly modulates cardiac activity during the postnatal period, which manifested at the level of effector structures in the heart and activity of regulatory systems (increase in noradrenergic influences on the heart).

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