Comparative Analysis of the Effects of Various Stress Factors at the Stage of Early Organogenesis on Heart Rate Variability in Pregnant Female Rats and Their Offspring

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We studied changes in the autonomic balance of heart regulation (by the parameters of heart rate variability) in non-pregnant female rats and rats on the days 10-11 of pregnancy on the next day after stress provoked by acute hypobaric hypoxia, intermittent normobaric hypoxia, or immobilization. The same parameters were assessed in 36-day-old offspring. In non-pregnant rats, the intermittent hypoxia resulted in a shift of the autonomic balance of heart regulation towards activation of the parasympathetic nervous system; in pregnant females, immobilization led to a shift of the autonomic balance towards the sympathetic nervous system. In the offspring, the changes also depended on the type of stress.

Key Words: *stress; early organogenesis; heart rate variability*

Early developmental disorders caused by various stress factors lead to delayed consequences persisting up to advanced age [8,14]. Early ontogeny is one of the most stress-reactive stages of pregnancy [6]. Stress exposure during this period can result in internal abortion, premature birth, physical abnormalities, mental retardation, and dysfunction of various organs and systems [8,12,13].

Under natural conditions, acute hypobaric hypoxia (AHH) can seriously affect the mother and progeny. Another type of hypoxia, intermittent normobaric hypoxia (INH), consists of repeated episodes of normobaric hypoxia alternating with episodes of normoxia and occurs much more frequently. This type of hypoxia is associated with many pathological situations, such as obstructive sleep apnea, chronic obstructive pulmonary disease, asthma, and pulmonary fibrosis. Long-term hypoxia develops when the oxygen capacity of the blood and oxygen pressure are normal, but blood flow rate and circulation decrease. This type of hypoxia may develop during immobilization, which is also an adequate model of emotional stress. The negative effects of these stress-mediated effects may appear in the maternal body and during postnatal ontogeny and involve changes in several physiological and biochemical parameters.

Changes in heart rate variability are important manifestations of stress-mediated effects, reflecting shifts in the balance of autonomic regulation (BAR) of the whole organism [10]. We previously demonstrated the effect of antenatal AHH on age-dependent dynamics of ECG parameters, but the comparative analysis of the effects of antenatal stresses with various etiologies have not yet been conducted [4].

The study is aimed at comparison of the effects of different types of prenatal stress during the period of early organogenesis on pregnant females and progeny by the parameters of heart rate variability.

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MATERIALS AND METHODS

The work was carried out in non-pregnant and pregnant nonlinear albino rats and their offspring of both sexes in accordance with ethical principles and regulations recommended by the European Science Foundation and Helsinki Declaration.

Non-pregnant and pregnant rats during early organogenesis (days 9 or 10 of gestation) were exposed to one of three types of stress: AHH (non-pregnant, *n*=15; pregnant, *n*=32), INH (non-pregnant, *n*=9; pregnant, *n*=22), and immobilization (non-pregnant, *n*=11; pregnant, *n*=20). For AHH modeling, the animals were placed in a pressure chamber for 1 min and the pressure was reduced to a level equivalent to 5% O_2 content in the inspired air. The animals were divided into low and highly resistant to hypoxia by the time to respiratory arrest (<5 and >10 min, respectively) [5]. For INH modeling, the animals were placed in a sealed chamber and air or gas mixture containing 10.5% O_2 and 89.5% N_2 where alternately (5 min) delivered for 2 h [14]. Immobilization was carried out for 6 h in special plastic containers (5.5×5.5×15.5 cm). The experimental and control females were deprived of food and water for 6 h.

In experimental series I, ECG in pregnant and non-pregnant females was recorded on the next day for 3 min after stress under conditions of free behavior. In series II, ECG was recorded in the offspring of experimental females (39 males and 32 females) and the control females (19 males and 18 females) under conditions of free behavior on postnatal day 36. This testing period was chosen because in rats it corresponds to puberty onset [7]. In all cases, intact non-pregnant (*n*=25) and pregnant (*n*=29) females and their progeny served as the control.

One day before ECG registration, electrodes (metal thumbtack 10 mm diameter and 7 mm tip length) were implanted subcutaneously on the back in the sacral (1 electrode) and scapular areas (2 electrodes). Sodium thiopental in a dose of 60 mg/kg (60 mg/ml solution) was used as the anesthetic. ECG was continuously recorded using Iscope software (created by D. D. Vorontsov). The record represented a graph of temporal dynamics of the analog signal sampled at a frequency of 273 Hz (*i.e.* the signal was recorded every 4 msec). The following indices of heart rate variability were calculated from ECG parameters using Spike-C3 and Intervals software (created by D. D. Vorontsov): stress index (SI) by the formula:

$$SI = \frac{A_{Mo}}{2 \times Mo \times \Delta X},$$

where A_{Mo} is mode amplitude, Mo is sample mode, and ΔX is RR-interval scatter in the sample; and index of parasympathetic tone (IPT) by the formula:

$$IPT = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n-1} (RR_i - RR_{i+1})^2},$$

where *n* is the number of *RR*-intervals in the sample and RR_i is the duration of the *i*-th *RR*-interval.

Increased stress index was regarded as a shift of heart BAR towards predominance of sympathetic regulation circuit and increased index of the parasympathetic tone attested to a shift towards predominance of parasympathetic circuit [3].

Statistical analysis of the results was performed using the nonparametric Mann–Whitney *U* test.

RESULTS

In experimental series I, comparison of heart rhythm variability parameters in pregnant and non-pregnant control rats revealed a significant increase in stress index and a significant decrease in the index of parasympathetic tone in pregnant rats, which attests to a BAR shift towards activation of the sympathetic contour (Table 1) and is consistent with published data on increased heart rate [15].

It can be assumed that activation of the sympathetic contour in pregnant females is a manifestation of stimulation of the cardiovascular system function due to increased metabolism during pregnancy. Activation of circulatory system activity in normal pregnancy can provoke a more potent reaction to the stressor, and more rapid resource depletion of the body during prolonged or very strong action [2,9].

Despite the fact that BAR in pregnant and non-pregnant rats initially differed, no post-stress changes in heart rate variability were detected in both groups. The exceptions were the effects of INH in non-pregnant rats and the effects of immobilization in pregnant rats (Table 1). In non-pregnant females after INH stress, the index significantly decreased, which can be interpreted as a shift of BAR towards suppression of activation of the sympathetic regulatory contour. Immobilization in pregnant rats induced opposite shifts: activation of the sympathetic and suppression of the parasympathetic regulatory contours (stress index significantly increased and the index of parasympathetic tone decreased). These variations of stress response in pregnant and non-pregnant females could be due to differences in the initial BAR state.

In contrast to the pregnant females, significant deviations from the control were observed in the offspring (II series) on postnatal day 36 (Table 2). In the offspring of low-resistant females, antenatal AHH shifted BAR towards activation of the sympathetic regulative contour. Stress index significantly increased in rats of both sexes and the index of parasympathetic

TABLE 1. Changes in Heart Rate Variability under Conditions of Free Behavior in Non-Pregnant and Pregnant Rats on the Next Day after INH and Immobilization (*M*±*m*)

Group		Stress index	Index of parasympathetic tone
Non-pregnant females	Control (*n*=25)	0.63±0.07	2.97±0.18
	INH (*n*=9)	0.27±0.03*	3.28±0.23
Pregnant females	Control (*n*=27)	1.07±0.10^{+}	2.32±0.12^{+}
	Immobilization (*n*=20)	1.61±0.22*	1.70±0.09*

Note. $p<0.05$ in comparison with: *control, $^{+}$non-pregnant females.

tone decreased in males. At the same time, in the male offspring of high resistant females, BAR shifted towards activation of the parasympathetic regulatory contour: stress-index decreased significantly and the index of parasympathetic tone increased.

Antenatal immobilization shifted BAR towards activation of the autonomic regulatory contour in male offspring (stress-index significantly increased), whereas in female progeny differences from the control were negligible (Table 2).

No significant differences in the parameters of heart rate variability from the control were found in the offspring after antenatal exposure to INH.

Published data suggests that BAR undergo shifts towards the sympathetic nervous system, accompanied by blood pressure increase and stimulation of heart function. In combination with symptoms of vascular endothelium dysfunction, these changes increased probability of cardiovascular system pathology, including systemic hypertension, myocardial infarction, *etc*. [11]. It can be hypothesized that the observed differences in BAR are related to different individual rates of maturation of heart regulation. According to current views, the development of the cardiovascular system and its regulatory structures is still going on in 36-day-old animals [1,4]. Antenatal stress could produce more or less pronounced modulatory effect on this process and led to diverse consequences.

Thus, during the early puberty, the shifts in heart rate variability parameters were more incident in male offspring and depended on the type of prenatal stress. We can assume that the identified postnatal changes in BAR regulation can significantly affect the overall level of stress reactivity in offspring subjected to antenatal stress of various etiologies in the early organogenesis.

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TABLE 2. Changes in Heart Rate Variability Parameters in 36-Day-Old Male and Female Rats after Antenatal Exposure to AHH or Immobilization (*M*±*m*)

Gender	Group	Stress index	Index of parasympathetic tone
Males	Control to AHH (*n*=6)	0.32±0.03	2.72±0.13
	AHH low resistant (*n*=7)	0.97±0.18*	1.75±0.10*
	AHH high resistant (*n*=6)	0.07±0.01*	10.42±1.47*
	Control to immobilization (*n*=7)	0.38±0.05	2.88±0.24
	Immobilization (*n*=6)	1.71±0.43*	2.10±0.49
Females	Control to AHH (*n*=6)	0.26±0.05	7.26±1.61
	AHH low resistant (*n*=7)	0.37±0.11	3.18±0.69*
	AHH high resistant (*n*=6)	0.13±0.06	7.81±1.44
	Control to immobilization (*n*=7)	0.17±0.03	5.45±0.72
	Immobilization (*n*=6)	0.20±0.03	5.10±0.82

Note. *$p<0.05$ in comparison with the control.

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