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RAPID COMMUNICATION

Late Effect of Early Hypoxic Disturbance in the Rat Heart: Gender Differences

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Summary

Perinatal hypoxemia may have serious long-term effects on the adult cardiovascular system and may lead to sex-dependent changes in cardiac tolerance to acute ischemia in adult life. The aim of the study was to answer the question whether gonadectomy of the male and female rats in the early phase of ontogenetic development affects the late effect of perinatal hypoxia. Pregnant Wistar rats were placed into a normobaric hypoxic chamber (12 % O_2) 7 days before the expected date of delivery. Newborn pups were kept in the chamber with their mothers for another 5 days after birth. After hypoxic exposure all animals were kept for 3 months in room air. Some of the pups were gonadectomized right after removal from the hypoxic chamber. Ventricular arrhythmias were assessed on isolated perfused hearts. Castration did not influence arrhythmogenesis in the adult normoxic or perinatally hypoxic female hearts. Nevertheless, the number of arrhythmias was decreased in perinatally hypoxic gonadectomized males. In conclusion, we have shown that perinatal normobaric hypoxia increased cardiac tolerance to acute ischemia in adult male rats; however, it had no late effect in females. Gonadectomy did not affect arrhythmogenesis in both normoxic and hypoxic female hearts, whereas in males significantly decreased the number of arrhythmias.

Key words

Perinatal hypoxia • Gender • Isolated rat heart • Ischemia/reperfusion injury

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The evidence that perinatal hypoxemia may have serious long-term effects on the adult cardiovascular system is increasing in the last years (Rohlicek *et al.* 2002, Li *et al.* 2003, 2004). This fact is in accordance with Barker´s concept of fetal and neonatal programming, which is based on epidemiological studies showing that perinatal pathogenetic factors may be linked with the development of adult cardiovascular diseases (Barker 2000). According to Li *et al.* (2003, 2004), prenatal chronic hypoxia increased the susceptibility of the 6-month-old male rat heart to ischemia/reperfusion injury. Nevertheless, prenatal hypoxia was without any effect on infarct size in young, 2-month-old male hearts. Using the model of perinatal exposure to daily intermittent hypobaric hypoxia (IHH) we have demonstrated that hypoxia changes the tolerance of the adult rat myocardium to acute ischemic damage; this phenomenon was gender-dependent (Netuka *et al.* 2006). The aim of the present study was, therefore, to answer the question whether gonadectomy of the male and female rats performed during early phase of ontogenetic development may moderate cardiac resistance to ischemia/reperfusion injury in adult animals.

Table 1. Coronary flow, perfusion pressure and heart rate of the isolated perfused hearts in normoxic and perinatally hypoxic control and gonadectomized male and female rats before ischemia and after the first min of ischemia and reperfusion.

	n	Coronary flow	Perfusion pressure (mmHg)			Heart rate (min^{-1})		
			stabilisation	ischemia 1 min	reperfusion 1 min	stabilisation	ischemia 1 min	reperfusion 1 min
Males								
Normoxic	17	13.1 ± 0.6	54.6 ± 4.1	74.2 ± 5.3*	57.6 ± 3.0	265 ± 9	283 ± 12	274 ± 19
Hypoxic	14	13.7 ± 0.6	58.6 ± 4.3	85.1 ± 11.2*	57.5 ± 2.9	237 ± 6	260 ± 12	249 ± 17
Gonadectomized normoxic	10	12.8 ± 0.9	51.9 ± 5.1	68.4 ± 8.3*	57.8 ± 6.2	272 ± 9	292 ± 19	269 ± 25
Gonadectomized hypoxic	11	12.8 ± 0.9	60.3 ± 8.2	83.6 ± 14.0*	61.4 ± 4.9	248 ± 10	256 ± 12	271 ± 18
Females								
Normoxic	11	11.4 ± 0.5	39.1 ± 2.6‡	53.3 ± 3.8*‡	45.9 ± 3.1‡	262 ± 7	269 ± 11	256 ± 19
Hypoxic	11	12.6 ± 0.5+	53.4 ± 5.3+	74.6 ± 7.3*+	59.9 ± 5.9+	259 ± 10	276 ± 19	287 ± 20
Gonadectomized normoxic	10	12.9 ± 0.8	52.3 ± 5.3	70.1 ± 6.9*	59.2 ± 5.5	263 ± 13	267 ± 15	276 ± 34
Gonadectomized hypoxic	8	13.0 ± 1.2	58.4 ± 8.0	87.1 ± 18.4*	60.1 ± 6.6	255 ± 10	269 ± 15	272 ± 35

n, number of animals. Values are mean ± SEM. * $P<0.05$ vs. pre-ischemic value, ‡ $P<0.05$ female vs. male, + $P<0.05$ hypoxic vs. normoxic.

The investigations were performed in accordance with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). For better simulation of perinatal hypoxic condition in clinical practice, perinatal normobaric hypoxia (NH) was used. Pregnant Wistar rats were placed into a normobaric hypoxic chamber (12 % O_2) 7 days before the expected date of delivery. Newborn pups were kept in chamber with their mothers for another 5 days after birth. After hypoxic exposure all animals were kept for 3 months in the room air. Some of the pups were gonadectomized immediately after removal from the hypoxic chamber using the deep ether anesthesia (Ošťádalová and Pařízek 1968). Sham surgery was performed under identical conditions as the gonadectomy. Ischemic arrhythmias were measured on the isolated hearts perfused according to Langendorff under constant flow with the non-recirculating Krebs-Henseleit solution. After 25-min stabilization, regional 30-min no-flow ischemia was induced by the LAD coronary artery occlusion (for details see Szarszoi *et al.* 2001). Ventricular arrhythmias during ischemic insult were assessed according to the Lambeth Conventions (Walker *et al.* 1988). The results are expressed as means ± S.E.M. One-way ANOVA or ANOVA for repeated measures and subsequent Student-Newman-Keuls test were used for comparison of differences between groups. Differences were assumed as statistically significant when $P<0.05$.

Body weight (BW) and heart weight (HW) of normoxic, perinatally hypoxic and gonadectomized adult female rats were significantly lower as compared with age-matched male animals. Gonadectomy significantly decreased BW in normoxic and hypoxic male rats, whereas in both groups of females BW was increased; there were no differences between normoxic and perinatally hypoxic gonadectomized rats. HW was lower in all female groups as compared to males. Perfusion pressure and heart rate before ischemia and during ischemia and reperfusion are summarized in Table 1. None of the hearts exhibited ventricular arrhythmias during the preischemic phase. Castration and perinatal hypoxia did not influence arrhythmogenesis in adult female hearts. On the other hand, the number of premature ventricular complexes occurring as singles, salvos and ventricular tachycardia as well as the total number of arrhythmias was significantly decreased in perinatally hypoxic and gonadectomized males (Fig. 1).

Our results have shown that strong insult in the neonatal period like castration and NH has no late effect

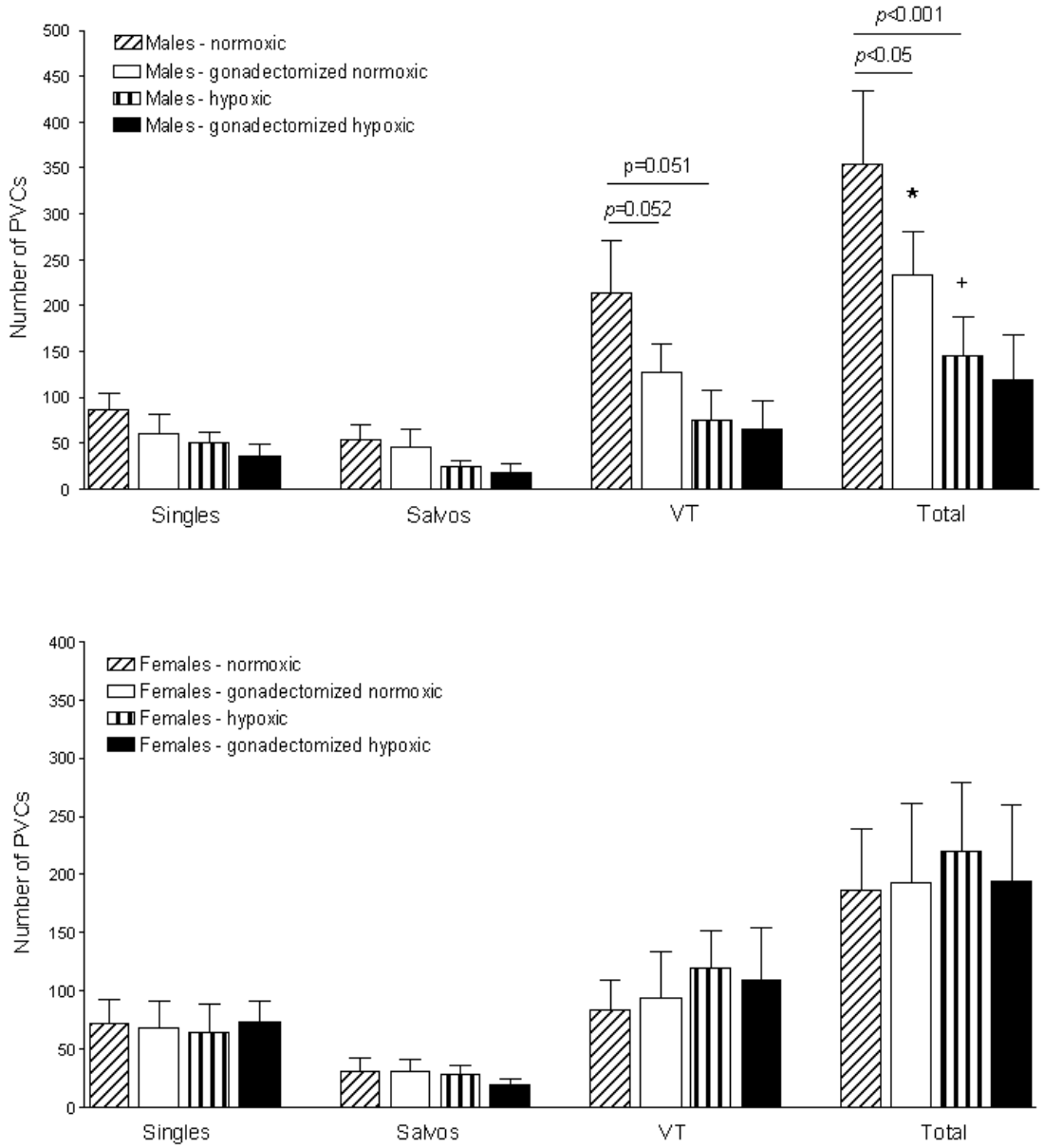

Fig. 1. Premature ventricular complexes (PVCs) occurring as singles, salvos and ventricular tachycardia (VT), total number of PVCs during 30-min coronary artery occlusion in the normoxic and perinatally hypoxic control and gonadectomized adult male (upper) and female (bottom) rats. Values are means ± SEM, * $P<0.05$ gonadectomized vs. corresponding control, $^{+}$ $P<0.05$ hypoxic vs. normoxic.

on the arrythmogenesis in adult females. On the other hand, gonadectomy as well as perinatal hypoxia significantly decreased ischemic arrhythmias in the adult males. Interestingly, in comparison with our previous study, the late effect of perinatal hypoxia on male and female myocardium is different. We have previously observed that perinatal exposure to IHH significantly increased cardiac tolerance to acute ischemic injury in adult females, expressed as the incidence of ischemic arrhythmias and reduced LDH. The effect of IHH on ischemic arrhythmias in males was quite opposite (Netuka *et al.* 2006). The explanation should probably be searched in the different types of hypoxia. Nevertheless, data from literature comparing the effect of normobaric and hypobaric hypoxia are very rare. Experimental studies focusing on late effects of early hypoxia used different types of hypoxia, e.g. Rohlicek *et al.* (2002) used hyperbaric hypoxia, whereas Li *et al.* (2003, 2004) and Hampl *et al.* (2003) used permanent NH. The comparison of the results in this situation (different types of hypoxic cycles and degree of hypoxia) is very difficult. Similarly, the data comparing intermittent and permanent hypoxia are poor. Farahani *et al.* (2008) have shown that postnatal IHH leads to significant body growth retardation and grater heart hypertrophy in comparison with permanent NH. Fan *et al.* (2005) observed that

apoptosis was enhanced in the neonatal mouse heart exposed to permanent hypoxia but not to intermittent hypoxia and this was correlated with an upregulation of proapoptotic genes and downregulation of anti-apoptotic genes in permanent hypoxia. In addition, there are only a few papers dealing with the gonadectomized male and female animals in the long-term effect of perinatal NH. Hampl and Herget (1990) and Hampl *et al.* (2003) found that ovariectomy at a very young age greatly augmented the effects of perinatal hypoxia in females, so that pulmonary arterial pressure in adulthood was significantly increased.

The question remains what is the cause of the gender difference in cardiac sensitivity to ischemia. A large body of evidence indicates that estrogen is involved in gender-related mechanisms of ischemia tolerance. Along with their well-known "genomic" effects, additional processes termed "non-genomic" occur rapidly and independently of protein synthesis (Di Lisa 2006). Among the many pathways that can modify the susceptibility to ischemic injury in female hearts is nitric oxide (Sun *et al.* 2006), sarcolemmal (Johnson *et al.* 2006) and mitochondrial K_{ATP} channels (Lee *et al.* 2000), protein kinase B (Akt) and protein kinase Cε levels (Bae and Zhang 2005) or tumor necrosis factor α (Xu *et al.* 2006). Nevertheless, the cardiovascular system is influenced not only by estrogens, but at least by one additional player – androgens. Similarly to estrogens, androgens are present in both sexes, albeit at different concentrations and ratios. However, the influence of testosterone on cardiovascular system is still controversial; experimental studies have shown its positive as well as negative effect on cardiac sensitivity to oxygen deprivation (for review see Ošťádal *et al.* 2009).

In conclusion, we have shown that perinatal exposure to NH increased cardiac tolerance to acute ischemic injury in adult male rats. However, permanent NH had no late effect in females. Gonadectomy did not affect arrhythmogenesis in both normoxic and hypoxic female hearts, whereas in males significantly influenced the incidence of arrhythmias in adulthood. Our results support the hypothesis that hypoxia in the early stages of ontogenesis is a programming stimulus leading to gender-dependent changes in cardiac tolerance to ischemia in later adult life.

Conflict of Interest

There is no conflict of interest.

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