Physiol. Res. 54: 357-362, 2005

Low Body Weight and Cardiac Tolerance to Ischemia in Neonatal Rats

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Received July 14, 2004
Accepted October 19, 2004
On-line available December 9, 2004

Summary

Adaptation to intermittent high altitude hypoxia (IHAH) increases tolerance of the isolated neonatal rat heart to ischemia and potentiates protection induced by ischemic preconditioning. In addition to the protective effect, IHAH significantly reduces growth of the animals. The aim of the present study was, therefore, to find out whether low body weight *per se* might influence cardiac sensitivity to oxygen deprivation. Low body weight was induced either by IHAH (barochamber, 8 h/day, 5000 m) from postnatal day 1 to 10 (HLBW), or by a higher number of sucklings per mother (14 instead of 8), again from postnatal day 1 to 10 (NLBW). Control animals (8 littermates per mother) were kept under normoxic conditions (Controls). The recovery of developed force following 40 min of global ischemia was measured in isolated hearts from 10-day-old rats by perfusing them in the Langendorff mode with Krebs-Henseleit solution at constant pressure, temperature and rate. Ischemic preconditioning was induced by three 3-min periods of global ischemia, each separated by 5-min periods of reperfusion. Low body weight in HLBW and NLBW groups was accompanied by increased hematocrit, and decrease in absolute heart weight (both wet and dry) and developed force. On the other hand, higher hydration, increased cardiac tolerance to ischemia and potentiation of protection by ischemic preconditioning were observed in HLBW rats only. This experimental group also exhibited the highest relative heart weight. It may be concluded that low body weight alone does not influence cardiac tolerance to ischemia in neonatal rats.

Key words

High altitude hypoxia • Low body weight • Cardiac tolerance to ischemia • Neonatal rats

Introduction

Cardiac tolerance to ischemia changes significantly during ontogenetic development. The immature mammalian heart is more resistant to oxygen deprivation than the adult heart (Riva and Hearse 1993, Ošťádalová *et al.* 1998), but the mechanisms of this difference have not yet been satisfactorily clarified (for review see Ošťádal *et al.* 1999). The interest of many experimental and clinical cardiologists during the past 40 years has been focused on the question of how cardiac tolerance to oxygen deprivation might be increased. We have observed that prenatal exposure of rats to intermittent high altitude hypoxia (IHAH) or ischemic preconditioning (IP) failed to increase cardiac tolerance to ischemia on postnatal day 1. On the other hand, both

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postnatal exposure to IHAH as well as IP improved recovery of developed force after ischemia on postnatal day 7 and 10 (Ošťádalová *et al.* 1998, 2002). Moreover, combination of IHAH and IP induced even higher protective effects as compared with both separate phenomena in all age groups under study, including postnatal day 1 (Ošťádalová *et al.* 2002).

Adaptation to IHAH is, however, accompanied by significant growth retardation (Ošťádal *et al.* 1984). In this connection it is necessary to mention that nutritional status markedly influences cardiac development. Slow-growing pups confer smaller cardiomyocyte length and volume (Bai *et al.* 1990), accompanied by qualitative changes of the subcellular structures. The development of membrane binding sites for α_1- and β-receptor ligands is retarded and the resulting receptor deficit probably contributes to reduced responsiveness to adrenergic stimulation (Bell and Slotkin 1988). These changes are connected with alterations of cardiac ornithine decarboxylase activity, starting within 48 h after modifying the litter size (Bell *et al.* 1987). Moreover, we have shown previously (Brodsky *et al.* 1992) that the number of cardiomyocytes in both the right and left ventricular myocardium of weanling rats was also dependent on litter size; a significantly lower number of cardiomyocytes was found in slow-growing as compared with fast-growing animals. Early postnatal nutritional modification also altered protein remodeling in the myocardium: concentration of collagenous proteins in slow-growing rats significantly decreased (Pelouch *et al.* 1997). Furthermore, undernutrition markedly decreased the basal values of left ventricular pressure and contractility in 3-week-old rats (Dowell and Martin 1984); data on younger animals are, however, lacking.

The aim of the present study was, therefore, to find out whether growth retardation *per se* might also influence cardiac tolerance to oxygen deprivation. For this purpose we have compared cardiac tolerance to ischemia in neonatal rats in which the low body weight was induced either by IHAH or by a higher number of littermates per mother.

Methods

All the investigations conform with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

Animal model

Fifty-six neonatal Wistar rats were used throughout the experiments. Animals born on the same day were pooled and assigned by random selection to dams in groups of 8 or 14 rats/litter. The first group (hypoxic low body weight - HLBW, 8 sucklings per litter) was exposed to IHAH simulated in a hypobaric chamber (altitude of 5000 m above sea level, barometric pressure 405 mm Hg, 54 kPa, PO_2 = 85 mm Hg, 11.3 kPa) 8 h/day, from postnatal day 1 to 9 (total of nine exposures). The second group (normoxic low body weight – NLBW, 14 sucklings per litter) as well as the third group (normoxic controls – Controls, 8 sucklings per litter) were kept for the corresponding period at barometric pressure and PO_2 equivalent to an altitude of 200 m above sea level. During the entire experiment, i.e. from postnatal day 1 to 10, littermates of all groups were kept at their mothers. The control as well as experimental groups were composed of at least three different litters. All mothers had free access to water and a standard laboratory diet *ad libitum*.

Assessment of heart function

On day 10 the animals were weighed and killed by cervical dislocation. Then the hematocrit was measured by a micromethod, the chest was quickly opened and a stainless steel cannula (with an external diameter of 0.8 mm) was inserted into the aorta. The heart was rapidly excised, the atria were removed and the ventricles were perfused in the Langendorff mode under constant pressure, corresponding to the mean arterial blood pressure for the given developmental stage, i.e. 73 cm H_2O (Litchfield 1958, Zicha *et al.* 1986). The hearts were perfused with a Krebs-Henseleit solution containing (in mmol/l): NaCl 118.0; KCl 4.7; $CaCl_2$ 1.25; $MgSO_4$ 1.2; $NaHCO_3$ 25.0; KH_2PO_4 1.2; glucose 7.0 and mannitol 1.1. The solution was saturated by a mixture of 95 % O_2 and 5 % CO_2 (pH 7.4) and temperature was maintained at 37 ^oC. The hearts were electrically stimulated at a rate of 200 beats/min using silver electrodes attached to the base of the heart. The stimulation was performed with pulses of alternating polarity, 1 ms duration and voltage set at 50 % above the threshold level. The resting force was gradually increased by means of a micromanipulator to the level at which the developed force (DF) was approximately 80 % of the maximum force reached at optimum preload. The contractile function of this isolated heart was measured using an isometric force transducer connected by a glass fiber, two-arm titanium lever and silk suture (0.7 metric)

to the apex of the heart. The DF (g) was evaluated automatically from the force signal using an on-line computer (Ošťádalová *et al.* 1993, 1996, 1998, 2002).

Experimental protocol

After a period of stabilization, baseline values of DF were recorded. One-half of the hearts from all three groups were preconditioned by subjecting them to three 3-min periods of global ischemia, each separated by a 5-min period of reperfusion. The remaining non-preconditioned hearts from all groups were simply perfused during the corresponding period. All hearts were then exposed to 40 min of sustained global ischemia followed by reperfusion up to maximum recovery of DF (the last value of DF before its decay) (Cave 1996, Ošťádalová *et al.* 1998). DF was measured in all hearts in 3-min intervals during the reperfusion period. The values of DF were expressed as a percentage of baseline values. After the experiment, the hearts were weighed; dry weight values were obtained after drying the tissue samples at 90 ^{o}C to constant weight.

Statistical analysis

The results are expressed as means ± SEM. Each observation was obtained from at least eight heart preparations in each group. Differences in the recovery of contractile function among the groups were evaluated using two- and one-way analysis of variance. For preliminary analysis, three-way analysis of variance was used. For pairwise mean comparisons the Student-Newman-Keuls multiple-range test was applied. All the used programs were from BMDP Statistical Software, University of California. Differences were considered statistically significant when $p<0.05$.

Table 1. Body and heart weight parameters.

Group	n	Body weight (g)	Hematocrit (%)	Wet heart weight (mg)	Heart/body weight (mg/g)	Dry heart weight (mg)	Dry heart weight (%)
Controls	13	23.7 ± 0.8	31.4 ± 0.5	100.5 ± 3.4	4.3 ± 0.1	19.2 ± 0.4	19.4 ± 0.7
HLBW	22	17.3 ± 0.4 [a]	39.5 ± 0.6 [ab]	85.0 ± 2.9 [ab]	4.9 ± 0.1 [ab]	15.0 ± 0.6 [ab]	17.7 ± 0.3 [ab]
NLBW	10	16.2 ± 0.8 [a]	34.3 ± 0.8 [a]	69.1 ± 2.8 [a]	4.3 ± 0.2	12.9 ± 0.4 [a]	18.7 ± 0.3

Significantly different ($p<0.05$): [a] from Controls, [b] from NLBW (normoxic low body weight)

Results

Weight parameters and hematocrit

Body and heart weight parameters and hematocrit are summarized in Table 1. Body weight in both experimental groups (HLBW, NLBW) was significantly decreased as compared to the controls. Wet and dry heart weights also decreased simultaneously; this effect was significantly more expressed in the NLBW group. Consequently, heart weight/body weight ratio and cardiac hydration were significantly higher in HLBW animals. Growth retardation was accompanied by increased hematocrit not only in the HLBW but also in the NLBW group.

Tolerance to ischemia

Absolute baseline values of DF were significantly decreased in both experimental groups as compared to the controls. DF of NLBW, expressed per g of dry heart weight, did not differ from the controls; it remained significantly higher than in the HLBW animals (Table 2). While exposure to IHAH significantly improved recovery of DF after ischemia, low body weight in normoxic animals was without any effect on the postischemic recovery of contractile function. On the other hand, a protective effect of IP was observed in all three groups under study. In addition, IP potentiated the cardioprotective effect of exposure to IHAH (Fig. 1).

Table 2. Baseline values of contractile parameters

Group	DF (g)	DF/wet heart weight (g/g)	DF/dry heart weight (g/g)
Controls	4.6 ± 0.3	46.6 ± 3.0	241 ± 16
HLBW	3.4 ± 0.1 [a]	41.0 ± 2.4 [b]	233 ± 14 [b]
NLBW	3.5 ± 0.2 [a]	50.0 ± 2.2	269 ± 10

DF – developed force. Significantly different ($p<0.05$): [a] from Controls, [b] from NLBW (normoxic low body weight)

Discussion

Weight parameters of slow-growing pups are in good agreement with previously published data (Bell and Slotkin 1988, Bai *et al.* 1990, Pelouch *et al.* 1997). Furthermore, our results demonstrate that tolerance of the neonatal rat heart to ischemia is not influenced by growth retardation *per se*, which is induced by malnutrition during the early phase of postnatal ontogeny. This suggests that at least in neonatal rats the cardioprotective effect of adaptation to IHAH or IP is not modified by growth retardation.

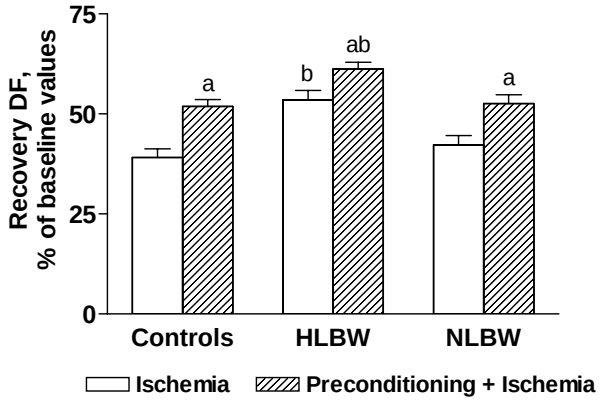

Fig. 1. Cardiac tolerance to ischemia in controls, hypoxic low body weight (HLBW) and normoxic low body weight (NLBW) rats. The effect of ischemic preconditioning. The data are means ± S.E.M. [a] Significantly different ($p<0.05$) vs. ischemic groups, [b] significantly different ($p<0.05$) vs. Controls and NLBW.

As mentioned in the Introduction, the mechanisms of cardioprotective effect of adaptation of the immature heart to chronic hypoxia and ischemic preconditining remain unknown. Although many potential factors have been proposed to play a role, the available data are not sufficiently conclusive (for review see Kolář *et al.* 2003, Kolář and Ošťádal 2004). Limited evidence exists for the involvement of K_{ATP} channels, reactive oxygen species, nitric oxide and protein kinases (Ošťádalová *et al.* 1998, 2002, Baker *et al.* 1999, Eells *et al.* 2000), but potential contributions of other factors cannot be excluded at present. Similarly, we cannot exclude that malnutrion during the early phases of ontogenetic development may influence cardiac sensitivity to oxygen deprivation in adulthood.

It was found that suckling rats fed in litters larger than normal grow more slowly and attain smaller body and heart weights. But what is the reason for growth retardation in IHAH-exposed animals? Rat pups less than 15 days old are exclusively dependent on the mother for nutrition, warmth, urination and defecation (Babický *et al.* 1970, 1973). It would, therefore, not be surprising that maternal IHAH-induced deprivation may negatively influence the quality and quantity of milk production. In addition, IHAH-induced depression of the suckling response to fasting cannot be excluded (Henning 1981). Some of the initial loss of body weight that is common for the exposure to altitude in adults is brought about by dehydration. Mild hypoxia induces polyuria and, in those who acclimatize well, there may be diuresis that lasts for days. With increasing altitude there is progressive dehydration due to the increased pulmonary ventilation induced by hypoxia (Heath and Williams 1995). During the exposure to high altitude the basal metabolic rate also increases (Nair *et al.* 1971) and this is ascribed to the stress of acute exposure to hypobaric hypoxia with associated sympathetic activity and stimulation of the adrenal cortex. And last but not least, it is likely that the basis for much of the initial loss of weight at high altitude is anorexia and hypophagia (Gloster *et al.* 1974).

Milk supplementation in neonates is the only source of calories and liquids. The higher hematocrit at NLBW is therefore probably induced by dehydration. On the other hand, the increase of hematocrit value at HLBW is also due to high altitude-induced polyglobulia (Ošťádal and Kolář 1999).

It may be concluded that growth retardation of neonatal rats, induced by the increase in litter size, has no effect on cardiac tolerance to oxygen deprivation. It seems that undernutrition is not involved in the cardioprotective effect of adaptation to chronic hypoxia and ischemic preconditioning during the early phase of ontogenetic development. On the basis of our results we cannot, however, exclude possible effects of altered nutritional status during the weaning period on cardiac tolerance to ischemia in adulthood.

Acknowledgements

This work was supported by research grant 305/00/1659 from the Grant Agency of the Czech Republic and LN 00A069 from the Ministry of Education, Youth and Sports of the Czech Republic. Preliminary results of this study were presented at the Young Investigator Workshop of the Center for Experimental Cardiovascular Research in Pec pod Čerchovem, September 21-22, 2003 (Chvojková *et al.* 2004).

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Reprint requests

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