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Ontogenetic Differences in Cardiopulmonary Adaptation to Chronic Hypoxia

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Summary

Cardiopulmonary adaptation to chronic hypoxia was compared in rats exposed to simulated high altitude (barochamber, 8 h per day, 5 days a week, stepwise up to 7000 m, a total of 24 exposures) either from the 4th day or the 12th week of postnatal life. Pulmonary hypertension and right ventricular (RV) enlargement were comparable in both age groups. Whereas in young hypoxic animals the individual values of RV weight increased linearly with a rise of RV pressure ($r=0.72$), no significant correlation was found in adult rats. Chronic hypoxia increased the concentration of cardiac collagenous proteins; this effect was more pronounced in adult animals. On the other hand, the collagen I/III ratio was markedly lower in young rats suggesting increased synthesis of collagen III in this age group. A protective effect of adaptation, i.e. increased cardiac resistance to acute hypoxic injury, was similar in both age groups and persisted even 4 months after removal of animals from the hypoxic atmosphere.

Key words

Ontogeny – Rat – Adaptation – Chronic hypoxia – Right ventricle – Pulmonary hypertension

Adaptation to chronic hypoxia is characterized by a variety of functional changes in order to maintain homeostasis with a minimal energy expenditure (Durand 1982). Such adjustment may protect the heart under conditions which require enhanced work and consequently increased metabolism. In contrast to the protective effect, adaptation to chronic hypoxia may, however, also exert an adverse influence on the cardiopulmonary system as evidenced in chronic hypoxia – induced pulmonary hypertension and right ventricular (RV) hypertrophy which may result in congestive heart failure.

Whereas relatively numerous studies are dealing with the effect of chronic hypoxia on the adult cardiopulmonary system (for review see e.g. Moret 1980, Ošťádal and Widimský 1985, Meerson 1990, Ošťádal *et al.* 1994), much less is known about the responses of the immature organism. The literary data are scarce and often contradictory. Different conclusions may be due to methodological variability: different models of hypoxia, the length and intensity of hypoxic exposure, anaesthesia, as well as the species, strain, sex and age of experimental animals. The present survey summarizes some of our results dealing with the ontogenetic differences in cardiopulmonary adaptation of rats to chronic intermittent high altitude (IHA) hypoxia.

Experimental Model

Adequate experimental models of chronic hypoxia are of crucial importance for a detailed study of the pathogenetic mechanisms participating in the development of human disease (for review see Herget and Paleček 1978, Ošťádal and Kolář 1989). It is necessary to mention that the different types of hypoxic situations which occur in man may be accompanied by other factors (e.g. cold, lowered air humidity, and radiation at high altitude). Furthermore, the lack of oxygen may affect not only the cardiopulmonary system but also many different organ and tissues (Bouveret 1985). Unfortunately, no existing model adequately reproduces the structural, functional and metabolic changes which occur in human pathology. This is not surprising if one considers the complexity of the conditions which have to be simulated in an experimental model. The tried and proven experimental model used in research on chronic

hypoxia is high altitude hypoxia either natural or simulated under laboratory conditions.

Chronic hypoxia is, however, not always permanent; it is often intermittent, e.g. in exacerbation of chronic obstructive lung disease during acute respiratory infection or during repeated ascents in mountains. Hypoxia is likewise not continual in myocardial ischaemia, when it depends on the actual coronary blood flow (Ošťádal and Widimský 1985). We therefore modified a model of chronic but intermittent hypoxic hypoxia, first described by Kopecký and Daum (1958) and Poupa *et al.* (1966), in which animals are exposed to a hypoxic environment for only a given part of the day. It has been shown in adult rats that not only permanent, but also intermittent hypoxia, simulated in a barochamber (4–8 h/day, 5 days a week, stepwise up to 7000 m) may stimulate both favorable cardiopulmonary adaptation as well as impose a stress the magnitude of which depends upon the intensity and duration of the hypoxic stimulus (Widimský *et al.* 1973, McGrath *et al.* 1973, Ošťádal and Widimský 1990).

Relationship between hypoxic pulmonary hypertension and right ventricular enlargement – effect of age

Cardiac enlargement may be the result of both an increase in the number of individual cell elements (hyperplasia) and an increase in their volume (hypertrophy). The participation of both processes in cardiac growth depends on the cell type and age of the animal. Whereas connective tissue cells can proliferate during the whole ontogeny, the mitotic activity of ventricular myocytes is limited to a relatively short postnatal period. In rats it ceases 4–6 weeks after birth, (Rakušan *et al.* 1965, Sasaki *et al.* 1970, Zak 1974). It was, therefore, of interest to establish whether cardiopulmonary responses to IHA just after birth will be different from those of adult male rats. Particular attention was paid to the ontogenetic difference in the relationship between pulmonary hypertension and RV enlargement (Kolář *et al.* 1989).

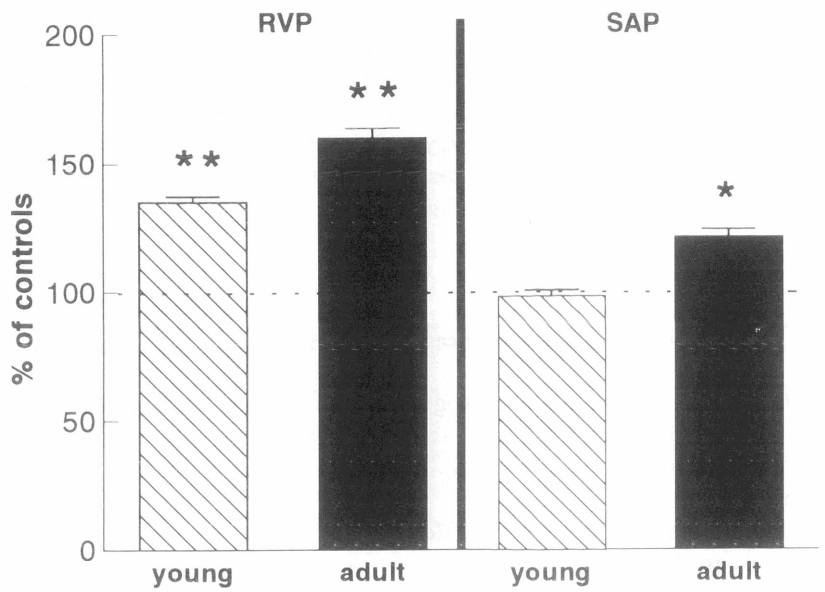

Fig. 1
Effect of intermittent high altitude hypoxia on right ventricular systolic pressure and systemic arterial pressure in young and adult rats; * – $p<0.01$; ** – $p<0.001$. Data from Kolář *et al.* (1989).

IHA hypoxia was simulated in a low pressure chamber for 8 h/day, 5 days a week. Barometric pressure was lowered stepwise so that the level equivalent to an altitude of 7000 m was reached after 13 exposures; the total number of exposures was 24. Two groups of Wistar male rats were exposed to IHA: "young" group already exposed from the 4th day of postnatal life (8 sucklings per litter with mother), "adult" group exposed after reaching the 12th week of life. The investigations were carried out within 24 h after the last exposure to IHA.

In adult rats, IHA induced a mild increase of heart rate and systemic arterial pressure (Fig. 1). This response is probably the consequence of a stress reaction, connected with the repeated alternation of hypoxia and normoxia under our experimental conditions. In rats exposed to permanent hypobaric hypoxia, the elevation of the systemic pressure was not observed (Rabinowitch *et al.* 1981). The same holds for the animals exposed to IHA from the 4th day of life. This ontogenetic difference may be considered as a consequence of the higher resistance of the developing organism to the influence of stress.

IHA induced chronic pulmonary hypertension (Fig. 1) and RV enlargement in both age groups. Whereas the pressure elevation in the lesser circulation was more expressed in adult hypoxic animals, the RV enlargement was higher in the young group of animals. RV weight increased linearly with a rise in pulmonary blood pressure in young hypoxic animals ($r=0.72$) (Fig. 2); this relationship was, however, very loose in adult rats ($r=0.16$).

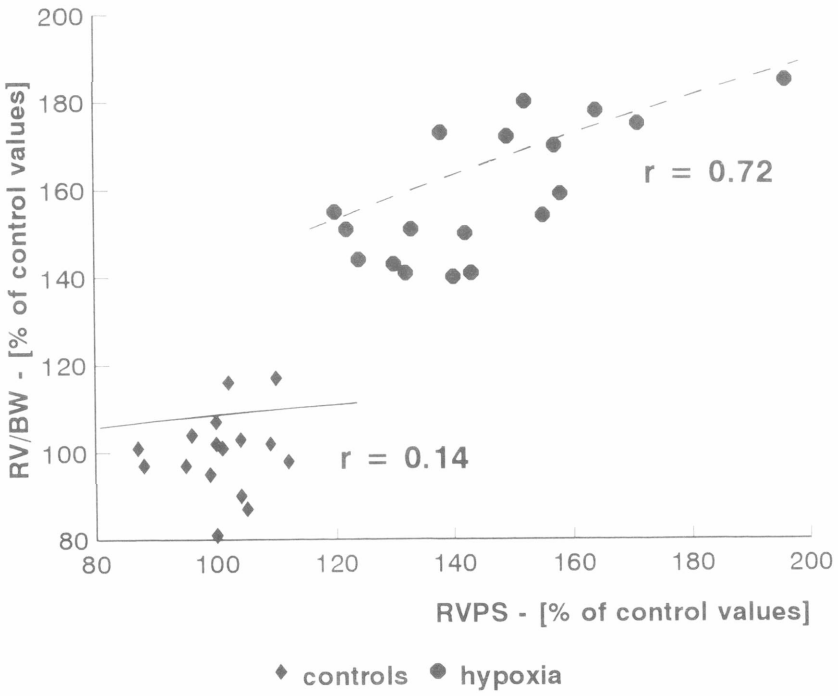

Fig. 2
Relationship between right ventricular systolic pressure (RVPS) and relative right ventricular weight (RV/BW), expressed as percentage of mean control values, in young controls (lower line) and young group exposed to intermittent high altitude (upper line). Data from Kolář *et al.* (1989).

The literature concerning the influence of age on chronic hypoxia-induced cardiopulmonary changes is insufficient and not concise. Smith *et al.* (1974) reported that young rats exposed to chronic hypobaric hypoxia showed a lower RV hypertrophy than adult animals, but the young group was not acclimatized before the 21st day of life. Rabinowitch *et al.* (1981) compared cardiopulmonary changes in rats exposed to permanent hypoxia (corresponding to an altitude of 5500 m) starting either from the 9th day of life or in adults. They found that the age difference in pulmonary hypertension and RV hypertrophy was not statistically significant.

Different results are obviously not only due to the different models of hypoxia (permanent vs. intermittent), but predominantly due to the different ontogenetic period of hypoxic exposure. It has been namely postulated that the most critical developmental period for myocyte proliferation and maturation in rats is limited to the first postnatal week (Brodsky *et al.* 1980, Ošťádalová *et al.* 1993, 1994). Hypoxic exposure in the above cited studies (from the 9th or even from the 21st day) thus seems to be too late for the significant stimulation of hyperplastic growth.

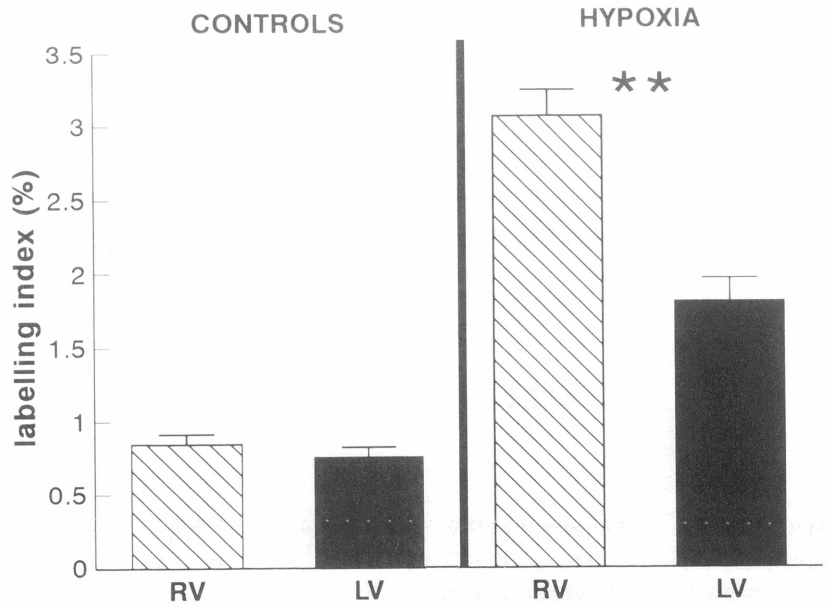

Fig. 3
The percentage of labelled cells in the right (RV) and left (LV) ventricular myocardium in control and intermittent high altitude-exposed rats; ** – $p<0.001$. Data from Wachtlová *et al.* (1977).

Although RV is the one exposed to the increased work load under conditions of chronic hypoxia, both ventricles are exposed to the same level of arterial hypoxia. Myocardial histoautoradiography in rats exposed to IHA on the 30th day of life (Fig. 3) showed that DNA synthesis in the muscle cell nuclei increases not only in the already enlarged RV but also in the non-enlarged left ventricle (LV) (Wachtlová *et al.* 1977, Arefyeva *et al.* 1985). The muscle cell proliferation may thus be stimulated either by hypoxia itself or by hypoxia-induced haemodynamic changes.

In this connection it is interesting to mention that chronic hypoxia also stimulates the proliferation of the so-called "pulmonary myocardium", the intrapulmonary bed of the pulmonary veins, encapsulated by a layer of cardiac musculature (Jarkovská and Ošťádal 1983). This enlargement occurs in the developing mice even at the time when the proliferation in the RV myocardium no more occurs. This difference may be explained by delayed morphogenesis of the pulmonary myocardium as compared with the proper heart (up to 9 days) (Klika and Jarkovská 1976).

A more detailed analysis of the relationship between the RV weight and pulmonary hypertension induced in different periods of postnatal ontogeny is still lacking. Our finding that in young animals a lower degree of pulmonary hypertension was connected with greater RV enlargement supports the opinion that the ventricular growth response to chronic hypoxia differs during development. The stimulus may be both the decrease of PO_2 and the secondary influence of altered haemodynamics. The close correlation between the two parameters in young hypoxic rats may thus be the consequence of the higher "reactivity" of the developing heart. Nevertheless, ontogenetic differences in other factors, participating in the regulation of circulatory homeostasis, cell growth and proliferation, have also to be taken into consideration.

Chronic hypoxia-induced developmental changes in cardiac protein profile

Development of chronic hypoxia-induced RV hypertrophy in adult rats is accompanied by significant changes in the protein profiling both in the hypertrophic RV and non-hypertrophic LV (Ošťádal *et al.* 1978). Right-to-left differences, characteristic for animals living in a normoxic environment, e.g. higher collagen concentration in the RV and higher concentration of myofibrillar proteins in the LV, did not change. We have observed a shift in cardiac isoform expression from the high affinity V_1 myosin ATPase to the lower activity V_3 form (Pelouch *et al.* 1985, 1993). IHA also modulates qualitative and quantitative changes of collagenous proteins. The proportion of this fraction significantly increased; simultaneously, the collagen I/III ratio decreased, suggesting and increased synthesis of collagen III (Fig. 4).

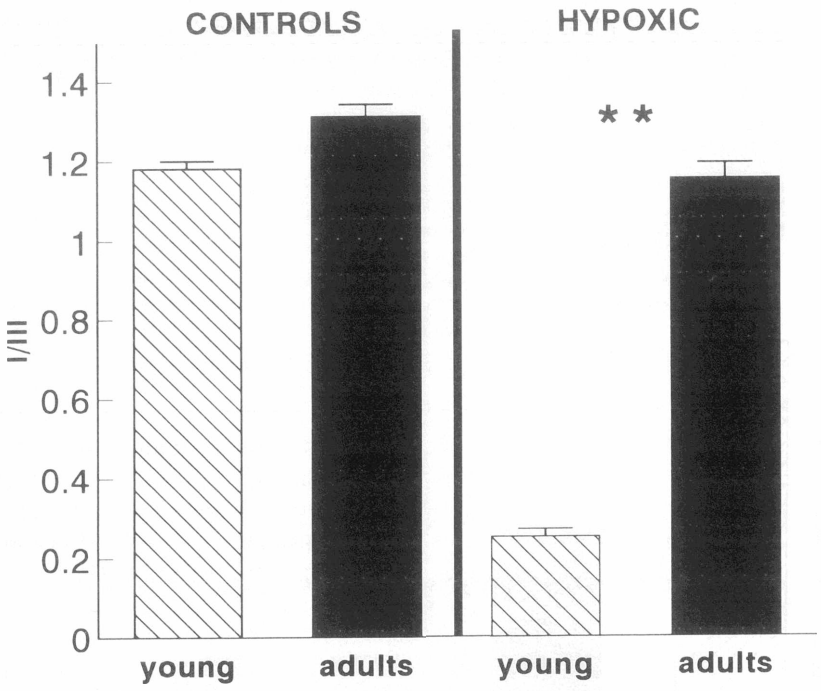

Fig. 4
Collagen I/III ratio in the right ventricle of young and adult animals exposed to intermittent high altitude; ** – $p<0.001$. Data from Pelouch *et al.* (1993).

In young animals, exposed to IHA from the 4th day of postnatal life, the concentration of contractile, collagenous and sarcoplasmic proteins increased in RV already after the 7th hypoxic exposure, when a RV enlargement was not yet observed. Whereas a higher concentration of sarcoplasmic and

collagenous fraction persisted even after the 24th exposure, the concentration of contractile proteins returned to the control values (Pelouch *et al.* 1987, 1993). IHA hypoxia delayed the transformation of isomyosin V_3 to V_1, which normally occurs during rat ontogeny (Lompre *et al.* 1981). This results in an increased proportion of isomyosin V_3, as it does in adult hypoxic rats or in other types of cardiac overload (Swynghedauw 1986). Such shifts are connected with decreased ATPase activity of the myosin molecule (Pelouch *et al.* 1987). It seems that these adaptive changes affect the thermomechanical economy of myocardial contraction. In young rats IHA increased both types of collagen, but the elevation of collagen III was significantly higher (Fig. 4). The functional significance of structural changes of collagen is, however, unclear.

Protective effect of adaptation to chronic hypoxia on the cardiac muscle in young and adult animals

In chronic high altitude hypoxia the myocardium must maintain adequate contractility in spite of lowered oxygen tension in the coronary circulation. Such an environment demands adaptation, which may protect the heart during conditions which require enhanced work. It was reported that the incidence of myocardial infarction is lower in people who live at high altitude (for review see Heath and Williams 1981). In addition to altitude hypoxia, the protective role of increased physical activity and reduced obesity has to be taken into consideration.

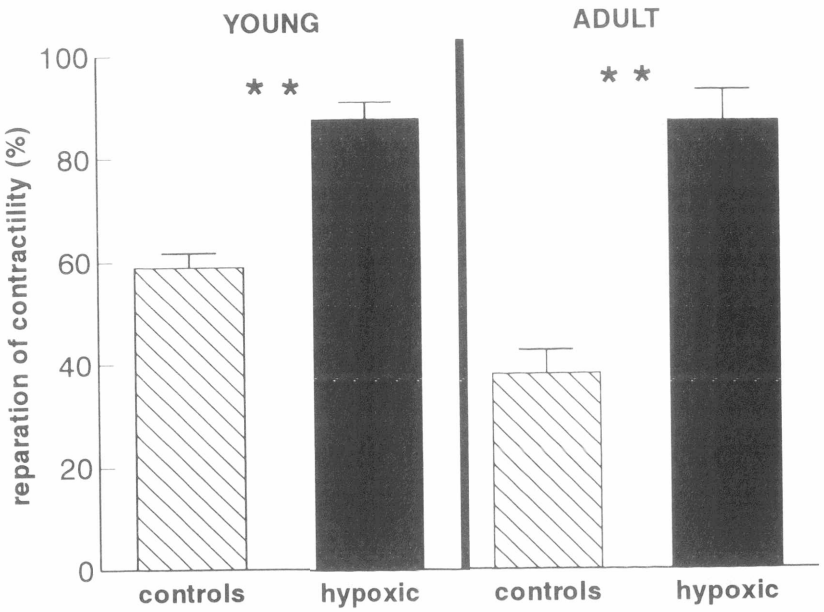

Fig. 5
Restitution of contractility of the isolated papillary muscle (expressed as percentage of recovery after nitrogen anoxia) in young and adult control animals and animals exposed to intermittent high altitude hypoxia; ** – $p<0.001$.

Epidemiological observations would be consonant with experimental studies using anoxia *in vitro*, necrogenic doses of isoprenaline and ligature of the coronary artery for testing the myocardial resistance in acclimatized animals. In this connection it is interesting to note that the first experimental studies on the protective effect of simulated high altitude on the isolated cardiac muscle (expressed as the recovery of isotonic contractions after nitrogen anoxia) were done in Prague (Kopecký and Daum 1958, Poupa *et al.* 1966). These findings were later confirmed by McGrath and Bullard (1968) and Meerson *et al.* (1973). We have observed (Widimský *et al.* 1973, McGrath *et al.* 1973) that the same protective effect can be induced by a relatively short exposure of rats to IHA.

The resistance of the isolated right ventricular wall to acute anoxia *in vitro* changes significantly during ontogenetic development. It increases from birth up to the 30th day (i.e. up to the end of weaning) in both male and female rats. From the 30th to the 60th day, however, this value decreased in males and remained constant in the female heart. The myocardium of control adult female rats thus proved to be more resistant to oxygen deficiency as compared with the adult male heart. IHA resulted in similarly enhanced resistance in rats exposed to hypoxia either from the 4th day of postnatal life or in adulthood (Fig. 5), yet the sex difference was maintained (Ošťádal *et al.* 1984). Cardiac resistance to acute anoxia remains higher even 4 months after the removal of animals from the hypoxic environment (Ošťádal *et al.* 1994)

The protective effect of adaptation to chronic hypoxia on the heart may be due to several mechanisms including e.g. an increased density of capillaries, increased content of myoglobin and mitochondria or an increased capacity of anaerobic metabolism (for review see Moret 1980). This view is supported by our findings in young and adult rats

acclimatized to IHA (Bass *et al.* 1989): chronic hypoxia induced comparable changes of energy metabolism in both age groups; no significant differences were found between the right and left ventricular myocardium. Glucose utilizing capacity (hexokinase) as well as the capacity for the synthesis and degradation of lactate (lactate dehydrogenase) were significantly increased. On the other hand, the ability to break down fatty acids (3-hydroxy-acyl-CoA-dehydrogenase) significantly decreased. However, the possible role of IHA-induced cardiac remodelling cannot be excluded: IHA-induced increase in the proportion of collagenous proteins may at least contribute to the lower oxygen requirement of the cardiac tissue. Still unclear is the role of sexual hormones as well as the optimal altitude and length of adaptation.

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