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Perinatal Lung Injury Extends in Adults the Site of Hypoxic Pulmonary Vasoconstriction Upstream

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Summary

Adult rats born in hypoxia but raised in air are more reactive to acute hypoxic challenges. The relation between perfusion pressure and perfusion flow (P/Q plot) was analyzed in the preparation of ventilated perfused lungs isolated from 3 groups of adult rats. Control animals of the first group were born and lived in air, the second group was born in hypoxic chamber and then the rats were raised in air. Rats of the third group were born in air and exposed to hypoxia in adulthood. The P/Q plot in rats born in hypoxia had lower slope than that in controls. Acute hypoxia in control group resulted in parallel shift of P/Q line to higher pressures. In rats born in hypoxia, however, both intercept with pressure axis and slope were increased. This may be explained by the participation of both collapsible and non-collapsible parts of pulmonary vascular bed in hypoxic pulmonary vasoconstriction. Analysis of distribution of pulmonary vascular resistances by the double occlusion technique confirmed this possibility. In rats born in hypoxia both arterial and middle vascular segment resistances increased during acute hypoxic challenge. In control rats, however, the increase in resistance was restricted to the middle segment only.

Key words
Hypoxic pulmonary vasoconstriction – Chronic hypoxia – Neonatal hypoxia – Pulmonary hypertension – Rat

Introduction

Hypoxic pulmonary hypertension is reversible within several weeks (Herget *et al.* 1978). Hypoxia applied in rats during the perinatal period, however, results in permanent changes of pulmonary vasculature (Hampl and Herget 1990, Hakim and Mortola 1990). Rats born in hypoxia but living in atmospheric air till adulthood were more reactive to acute hypoxic stimuli while they are recovering from the additional period of chronic hypoxia applied in adulthood (Hampl and Herget 1990). Similar permanent alterations of pulmonary blood vessels and enhancement of hypoxic vascular reactivity were found in adult rats who experienced prenatal pulmonary hypertension induced by injection of indomethacin to their mothers (Herget *et al.* 1994). The present study was focused on the possible mechanisms of the described permanent effects of perinatal hypoxia.

Methods

We measured the perfusion pressure-flow plots in normoxia and acute hypoxia (Herget *et al.* 1994) in preparations of isolated perfused lungs of adult rats (Herget and McMurtry 1985). The lungs were perfused with blood from rat blood donors. Then, using the double occlusion technique (Hakim *et al.* 1982), we partitioned the reactivity of different segments of pulmonary vasculature to acute hypoxic challenges.

Three groups of rats were used in this study. Rats in the experimental group PH (n=7) were born in a hypoxic chamber (F_{iO2}=0.12) and then kept in atmospheric air (Hampl and Herget 1990). They were compared with rats born in normoxia and exposed to chronic hypoxia (F_{iO2}=0.1, 3 weeks) at the age of 100–110 days (group AH, n=6) and with control rats who lived in atmospheric air throughout life (group CONTR, n=5). All three groups of rats were examined at the age of 120–140 days.

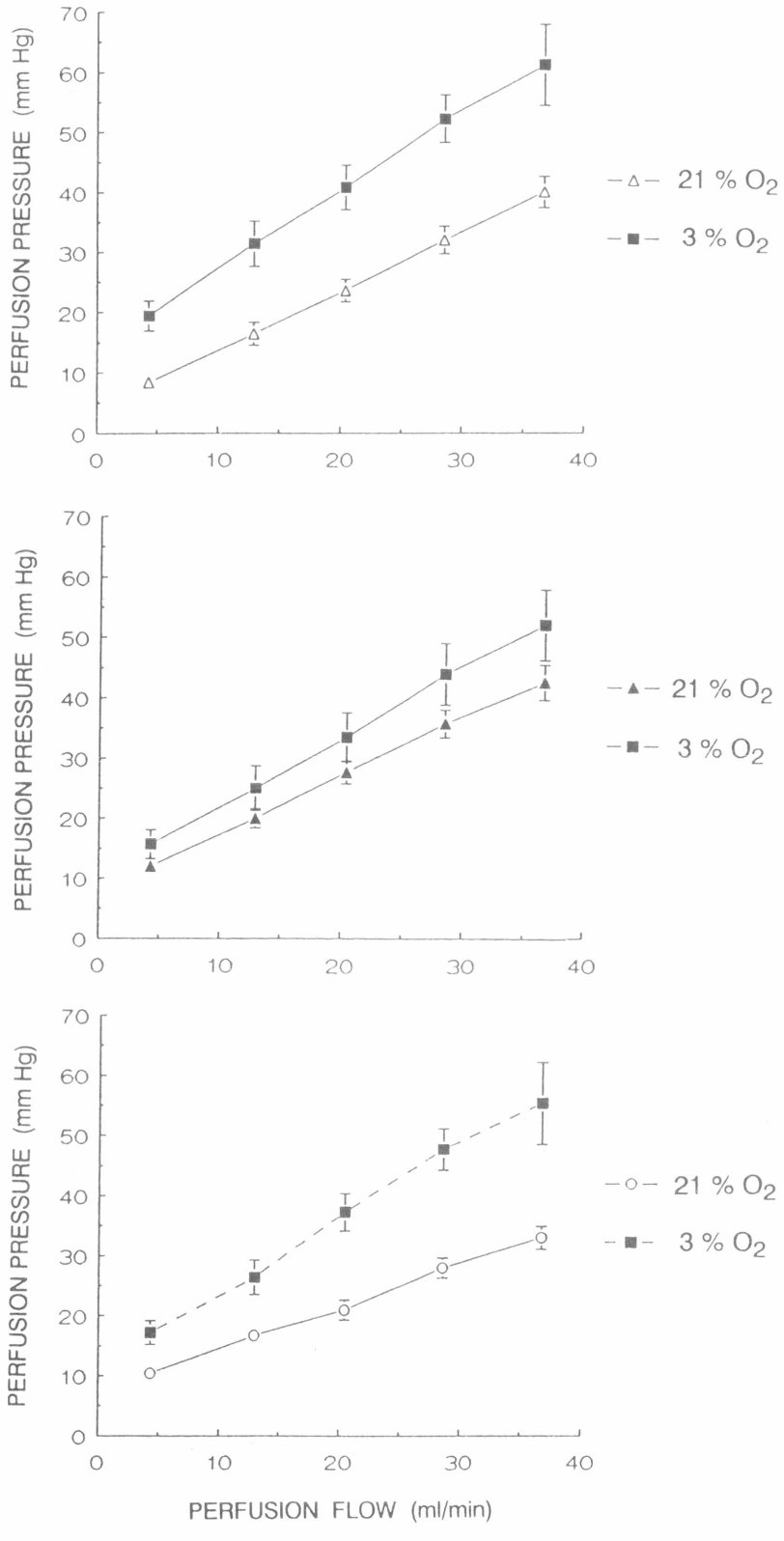

Fig. 1
Pressure-flow plot in isolated lungs of the control rats (upper panel), rats exposed to hypoxia in adulthood (middle panel) and rats born in hypoxia (lower panel). The increase of perfusion pressure was induced by stepwise increases of perfusion flow (in 5 min intervals). Data are means ± S.E.M. Two-way ANOVA indicates a significant parallel shift of P/Q line by acute hypoxic challenge in the control group, no significant change in rats exposed to hypoxia in adulthood and there was a significant upward shift and significant increase of slope of the P/Q line in rats born in hypoxia.

Results

There was no mortality in either group. On the day of the experiment, the body weight of rats in control group was 408±17 g, in group AH it was 312±21 g and in group PH 397±44 g. The differences in the body weight between groups were not significant.

The baseline perfusion pressure at the beginning of lung perfusion after stabilization of the preparation was 15.2±1.0 mm Hg in control group, 25.7±1.6 mm Hg in group AH (significantly higher than in groups CONTR and PH) and 13.7±1.5 mm Hg in group PH (not different from control group).

Table 1
Slope and intercept with pressure axis of the P/Q lines

Group	21 % O_2		3 % O_2	
	P/Q	P_o	P/Q	P_o
CONTR	0.95±0.07	3.79±0.72	1.11±0.19	21.16±3.42*
AH	1.34±0.06$^{\times}$	15.20±2.00$^{\times}$	1.44±0.14	15.15±1.80
PH	0.74±0.04$^{\times}$	7.19±0.68	1.27±0.10*	12.60±1.85*

** significantly different from respective value at 21 % O_2 (paired T-test), # significantly different from control group (CONTR) (ANOVA and Scheffé test), P/Q = slope of the PQ line, P_o = intercept with pressure axis, 21 % O_2 = lungs ventilated with 21 % O_2 + 5 % CO_2 balanced with N_2, 3 % O_2 = lungs ventilated with 3 % O_2 + 5 % CO_2 balanced with N_2. The relations of perfusion pressure-flow in individual rats were linear (correlation coefficients r>0.9732).*

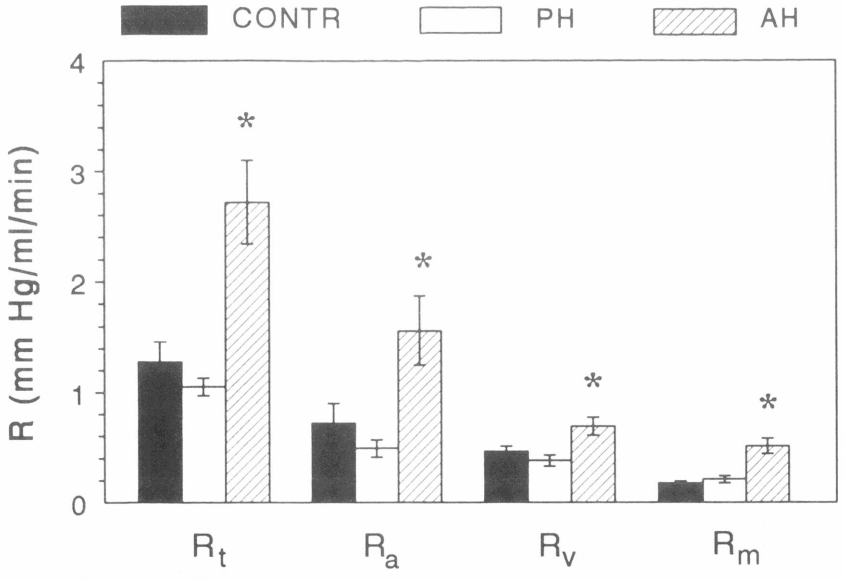

Fig. 2
Distribution of pulmonary vascular resistances in rats born in hypoxia (PH), rats exposed to hypoxia in adulthood (AH) and in the controls (CONTR). R_t = total pulmonary vascular resistance, R_a = resistance of arterial vascular segment, R_m = resistance of middle vascular segment, R_v = resistance in the venous vascular segment. * significant increase of resistance compared to CONTR and PH ($P<0.05$, ANOVA).

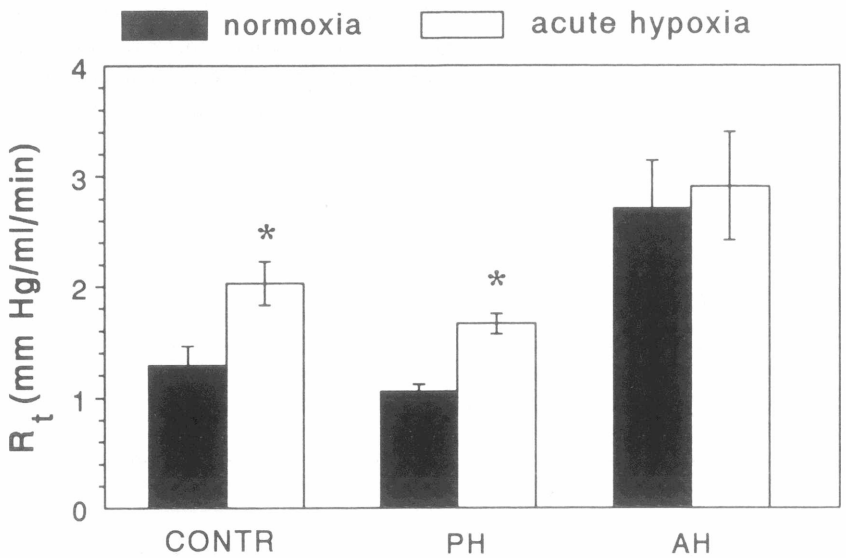

Fig. 3
Changes of total pulmonary resistance induced by acute hypoxia in rats born in hypoxia (PH), rats exposed to hypoxia in adulthood (AH) and in the controls. R_t = total pulmonary vascular resistance. * $P<0.05$, paired T-test.

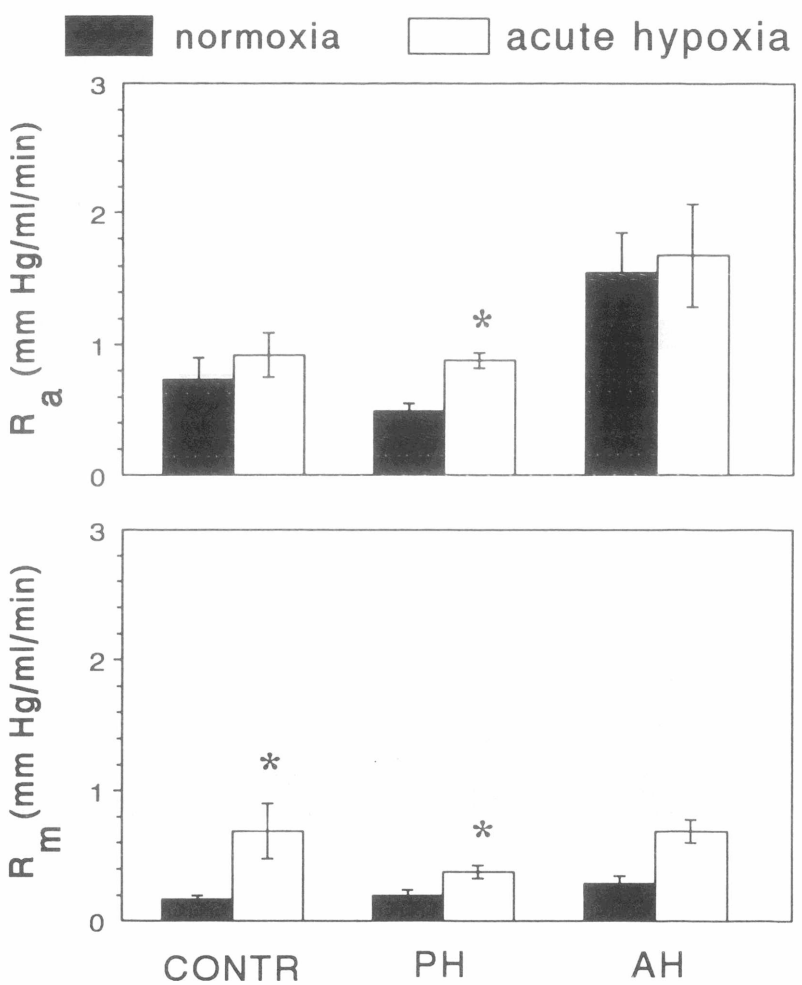

Fig. 4
Changes of pulmonary vascular resistances in arterial and middle vascular segments induced by acute hypoxia in rats born in hypoxia (PH), rats exposed to hypoxia in adulthood (AH) and in the controls. * $P<0.05$, paired T-test. R_a = resistance of arterial vascular segment (upper panel), R_m = resistance of middle vascular segment (lower panel).

Perfusion pressure-flow (P/Q) relationships

Exposure to hypoxia in adulthood (group AH) resulted in an upward parallel shift of the perfusion plot and the slope of P/Q line was steeper. Perinatal hypoxia influenced the slope of the P/Q curve, while the intercept with the pressure axis was not significantly different from that in the control group (Table 1). Acute hypoxic challenges in the control group led to a parallel upward shift of the pressure flow line (Table 1, Fig. 1, upper panel), the rats of group AH did not react to acute hypoxia significantly (Table 1, Fig. 1, middle panel). In contrast to the findings in the control group, acute hypoxia in rats born in a hypoxic chamber (group PH) both the intercept with the pressure axis and also the slope of the pressure-flow line were affected (Table 1, Fig. 1, lower panel).

Distribution of the pulmonary vascular resistances

We found in the occlusion experiments on adult rats exposed to chronic hypoxia (group AH) that the resistances in all parts of the pulmonary vasculature (total, arterial, middle and venous) were significantly higher than those in the controls and in rats born in hypoxia (PH) (Fig. 2). Acute hypoxic challenges increased total pulmonary vascular resistances in groups CONTR and PH. The lungs from animals of group AH did not react (Fig. 3). In the control group the increase of resistance was localized in the middle segment (Fig 4, lower panel). In rats born in hypoxia, however, the hypoxic pulmonary vasoconstriction was also localized in the arterial segment (Fig. 4). Acute hypoxia did not influence the resistance of the venous segment in either group.

Discussion

We have found that adult rats born in a hypoxic environment reacted differently to acute hypoxic challenges than adult rats born in normoxia. Their hypoxic pulmonary vasoconstriction also differed from that in rats born in normoxia but exposed to hypoxia in adulthood. Measurement of the perfusion pressure-flow relationship over a wide range of flow is suitable for estimation of the changes in pulmonary vascular resistance. This can be described by extrapolated pressure axis intercept at zero flow (P_O) and slope of the P/Q curve (P/Q). The first parameter represents the mean of critical closing pressures in pulmonary vasculature, whereas the second one is related to the upstream incremental vascular resistance. Critical closing pressure reflects the pressure balance at the walls of the middle collapsible portion of pulmonary vasculature. Upstream incremental resistance is the pressure expenditure to increase the flow in vessels in the upstream non-collapsible portion of the pulmonary vascular bed.

The P/Q plot in lungs isolated from rats exposed to hypoxia in adulthood (group AH) was significantly shifted upwards and it was steeper than that in the controls. This finding is in accord with the results obtained by the Sheffield group in rats exposed to hypoxia (Wach *et al.* 1987, Emery *et al.* 1981). The increased slope of the P/Q line in chronic hypoxia results from the increase of haemodynamic resistance of pulmonary vasculature by encroachment of the smooth muscle into the lumen of peripheral pulmonary arteries. The diameter of peripheral pulmonary vessels is decreased after chronic hypoxia (Finlay *et al.* 1986). The number of affected vessels may vary between species and a similar change of slope of the P/Q line was not found in pigs (Mitzner and Sylvester 1981). The increase in the slope of the P/Q line corresponds well with our finding of a higher resistance in the arterial segment of rats in group AH assessed by the occlusion technique. The slope of the P/Q line in rats born in hypoxia (group PH) was significantly lower than that in the controls. The resistances of the arterial segments in groups PH and CONTR, however, did not differ significantly. The lower slope of the P/Q plot may correspond to a higher compliance of the vascular wall or a greater cross-sectional area of the pulmonary vascular bed (Permutt and Riley 1963). Exposure of adult rats to chronic hypoxia results in a decrease in pulmonary vascular compliance (Finlay *et al.* 1986) due to the deposition of collagen and the growth of smooth muscle cells in the vascular wall (Hislop and Reid 1976). It seems unlikely that the perinatal hypoxia in our rats would have an opposite effect. A more probable explanation is that the cross-sectional area of the pulmonary vascular bed is increased. Lung injury in perinatal period which was associated with pulmonary hypertension (Albeda 1990) may enhance the genesis of pulmonary peripheral vessels (Folkman 1986).

The increase of the pressure axis intercept in group AH reflects the increase of the mean critical closing pressure (Dawson *et al.* 1989) due to the remodelling of the pulmonary vascular wall and increase of tone in the muscular arterioles in the alveolar region (Barer *et al.* 1987).

Acute hypoxic challenge in adult animals usually results in a parallel shift of the P/Q plot (Mitzner and Sylvester 1981, Hakim *et al.* 1983, Wach *et al.* 1987, Barer *et al.* 1987). It was shown by the occlusion technique that the increase of vascular resistance in acute hypoxia is located in the middle segment (Hakim *et al.* 1983). This is in agreement with our data obtained in the controls. The hyporeactivity to acute hypoxia in rats measured immediately after exposure in the hypoxic chamber was discussed elsewhere (Hampl and Herget 1990). In rats of group PH, however, both the slope and pressure axis

intercept of the P/Q line were increased. One of the explanations is that both collapsible and non-collapsible parts of the pulmonary vascular bed are constricted. It may be due to the fact that either new non-collapsible vessels, reacting to acute hypoxia were formed or a part of the originally collapsible vessels had lost their collapsibility but the responsiveness to hypoxia had been preserved. Loss of their collapsibility may result from an increased amount of connective tissue in the vascular wall or from smooth muscle cell proliferation. Our finding that acute hypoxia increased the resistances in both middle and arterial vascular segments in rats born in hypoxia supports this idea.

We believe that perinatal hypoxia induces an extension of the site of hypoxic pulmonary vasoconstriction to the upstream non-collapsible part of the vascular bed. This phenomenon induced in rats at birth continues throughout adulthood and may cause the hyperreactivity to acute hypoxic stimuli observed in our previous paper (Hampl and Herget 1990).

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