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Perinatal History of Hypoxia Leads to Lower Vascular Pressures and Hyporeactivity to Angiotensin II in Isolated Lungs of Adult Rats

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Summary

The most dramatic changes in pulmonary circulation occur at the time of birth. We hypothesized that some of the effects of perinatal hypoxia on pulmonary vessels are permanent. We studied the consequences of perinatal exposure to hypoxia (12 % O_2 one week before and one week after birth) in isolated lungs of adult male rats (~12 weeks old) perfused with homologous blood. Perfusion pressure-flow relationship was tilted towards lower pressures in the perinatally hypoxic as compared to the control, perinatally normoxic rats. A non-linear, distensible vessel model analysis revealed that this was due to increased vascular distensibility in perinatally hypoxic rats (4.1±0.6 %/mm Hg vs. 2.3±0.4 %/mm Hg in controls, $P = 0.03$). Vascular occlusion techniques showed that lungs of the perinatally hypoxic rats had lower pressures at both the pre-capillary and post-capillary level. To assess its role, basal vascular tone was eliminated by a high dose of sodium nitroprusside (20 μM). This reduced perfusion pressures only in the lungs of rats born in hypoxia, indicating that perinatal hypoxia leads to a permanent increase in the basal tone of the pulmonary vessels. Pulmonary vasoconstrictor reactivity to angiotensin II (0.1-0.5 μg) was reduced in rats with the history of perinatal hypoxia. These data show that perinatal hypoxia has permanent effects on the pulmonary circulation that may be beneficial and perhaps serve to offset the previously described adverse consequences.

Key words

Perinatal hypoxia • Vascular pressure • Pulmonary circulation • Angiotensin II

Introduction

Unlike the systemic circulation, pulmonary vessels are subject to an abrupt and intense change at the time of birth. Within seconds or minutes after birth, pulmonary blood flow increases from a small fraction of the cardiac output to its full value. At the same time, there is a several-fold decrease of pulmonary arterial blood pressure (PAP). Pulmonary vascular smooth muscle tone drops and a large-scale pulmonary vascular remodeling takes place (Haworth 1995).

These uniquely dramatic changes in pulmonary circulation at the time of birth led us to the hypothesis that pulmonary vessels may be more vulnerable to various external stimuli during the perinatal period than at other times (Herget *et al.* 1987, Hampl and Herget

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1990). Specifically, we had hypothesized that stimuli, such as chronic hypoxia, which have reversible effects on the pulmonary circulation in the adult, will alter the pulmonary vessels permanently if applied at the perinatal period.

This hypothesis was first tested using male rats exposed perinatally to 2 weeks of hypoxia (1 week *pre* and 1 week *post partum*) and then raised to maturity and studied in room air (Hampl and Herget 1990, Herget and Hampl 1990). One aspect in which the rats born in hypoxia were found to differ from controls was the pressure/flow relationship. It was tilted towards lower pressures in lungs of adult rats born in hypoxia as compared to perinatally normoxic ones (Fig. 1). Linear regression of the pressure/flow data yielded a reduced slope of the regression line in the perinatally hypoxic group (Herget and Hampl 1990, Herget and Kuklík 1995). The pressure axis intercept was elevated in one of our studies (Herget and Hampl 1990) but not in another (Herget and Kuklík 1995).

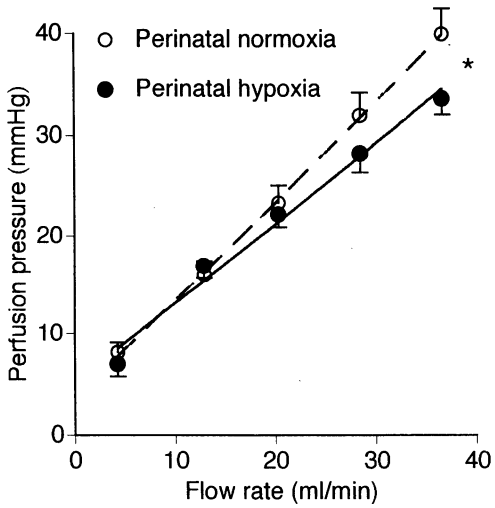

Fig. 1. *Perinatal experience of hypoxia tilts the perfusion pressure/flow relationship in lungs isolated from adult rats towards lower pressures. The data were obtained under zone 2 conditions (alveolar pressure 2.5-9 cm H_2O, outflow pressure –2 cm H_2O) and analyzed by linear regression. Individual values are group means ± S.E.M., lines are linear regressions for each group, n = 5 in the perinatally normoxic and n=7 in the perinatally hypoxic group. * The difference between the pressure/flow curves is significant (P < 0.05 by repeated measures ANOVA).*

While linear regression fits the pulmonary pressure flow curves well, a meaningful interpretation of its two parameters, the slope and pressure axis intercept, in physiological terms is uncertain (Linehan *et al.* 1992). Traditionally, so-called Starling resistor model has been used for this purpose, in which the pressure axis intercept is interpreted as a critical closing pressure for the collapsible segment of the pulmonary microvasculature. The slope corresponds to the resistive properties of the vessels upstream from the collapsible segment (Mitzner and Huang 1987). Using this reasoning, the reduced slope of the pressure/flow relationship in the perinatally hypoxic group could have been due either to a larger cross-sectional area or enhanced distensibility of the pulmonary vessels (Herget and Hampl 1990).

The Starling resistor model has several serious limitations (Linehan *et al.* 1992). One of them concerns the anatomical vagueness of the division between collapsible and non-collapsible vessels. In order to derive more meaningful information from the pulmonary perfusion pressure/flow data, Linehan *et al.* (1992) developed an alternative method based on the collapsible vessel model of the pulmonary circulation (Fung 1994). We decided to use this approach to gain a better understanding of the changes induced in the pressure/flow relationship by perinatal hypoxia. One of the main advantages of this method is that the analysis yields only two parameters, both with a clear physiological meaning. One parameter, α, represents vascular distensibility. The other, R_0, corresponds to vascular resistance at near zero flow, which is directly proportional to the resting cross-sectional area of the pulmonary vasculature (Linehan *et al.* 1992). A preliminary account of this work has been presented (Hampl *et al.* 1999).

Methods

Experimental groups

As in our previous studies (Hampl and Herget 1990, Herget and Kuklík 1995), adult male rats were studied which had been exposed to normobaric hypoxia (12 % O_2) for the last prenatal (*in utero*) and the first postnatal week. Control rats were born in room air. All animals were raised in room air and studied at the age of 10-14 weeks (Fig. 2).

Supplementary experiment

To see whether the alterations in the pressure/flow curves were due to hypoxia experienced specifically during the perinatal period or rather just delayed effects of hypoxia at any age, another group of

male rats was included in the study. These animals were born and raised in room air until 14 weeks of age, when they were exposed to 2 weeks of normobaric hypoxia (12 % O_2). They then recovered for 10-14 weeks in room air before they were used for the measurements (Fig. 2).

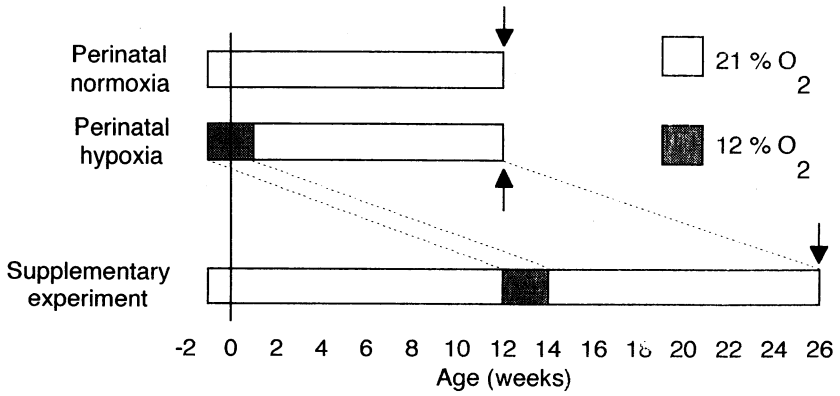

Fig. 2. *The upper two bars correspond to the two experimental groups in our main experiment. The uppermost bar represents control rats born and raised in room air throughout the experiment (n = 8). The second bar from the top indicates the perinatally hypoxic group, which was exposed to hypoxia 1 week before and 1 week after the birth and then raised in room air till maturity (n = 8). The bottom bar shows the additional group used in the supplementary experiment. These rats were subjected to the same hypoxic exposure followed by a long recovery as the perinatally hypoxic group. The only difference was that the hypoxic exposure did not start in the perinatal period, but rather after the rats had reached maturity (n=5). Arrows indicate the day of sacrifice.*

Isolated lungs

Isolated, blood-perfused lungs were prepared as previously described (Hampl and Herget 1990, Hampl *et al.* 1993, 1996). The rats were anesthetized with an intraperitoneal injection of thiopental (40 mg/kg body weight) and mechanically ventilated with air through tracheostomy (60 breaths/min; peak inspiratory and expiratory pressure of 10 and 0 cm H_2O, respectively). The chest was then opened and the main pulmonary artery and left atrium were cannulated. Using a peristaltic pump, the lungs were perfused with blood (hematocrit 41.2±0.7 %) obtained by cardiac puncture from 2-3 normal rat donors lightly anesthetized with ether. The gas mixture used for ventilation was switched from room air to 21 % O_2 + 5 % CO_2 + 74 % N_2. The lungs were excised from the body, suspended on the trachea in a heated (38 °C) humid chamber, and allowed to stabilize for 15 min at a perfusion flow rate of 12 ml/min and peak end-expiratory pressure of 2 cm H_2O. The outflow from the left atrial cannula was recirculated into the perfusate reservoir. The outflow pressure was set to –2 mm Hg.

Pressure/flow measurement and analysis

Immediately before the measurement of the pressure/flow relationship, the conditions of the lung were changed to zone 3 because the non-linear model of Linehan *et al.* (1992) was developed for zone 3. The positive end-expiratory pressure was removed and ventilation was stopped at the end of expirium. Outflow pressure was raised to +2.5 mm Hg. The flow rate of the perfusion pump was then changed in 30-second steps from the baseline rate of 12 ml/min to 2, 3, 4, 5, 10, 15, 20 and 25 ml/min. The resulting values of perfusion pressure were recorded and plotted against the respective value of flow rate.

The pressure-flow data were analyzed using the non-linear model of Linehan *et al.* (1992):

$$Pa = \frac{[(1 + \alpha Pv)^5 + 5\alpha R_0(Hct)Q]^{1/5} - 1}{\alpha}$$

In this equation, Pa is the measured inflow pressure, Pv is the outflow pressure maintained at +2.5 mm Hg, Q is the flow rate controlled by the perfusion pump, and Hct is hematocrit of the perfusate. As mentioned above, α is a parameter corresponding to vascular distensibility, and R_0 is a parameter corresponding to the resistance at near-zero flow (Linehan *et al.* 1992). The equation was solved for each pressure/flow curve separately using commercial computer software (Jandel SigmaPlot 3.0).

Sodium nitroprusside

The contribution of basal vascular smooth muscle tone to the pulmonary pressure/flow characteristics was studied by removing all tone by a high dose of sodium nitroprusside (20 μM) and repeating the pressure/flow measurement.

Occlusion methods

The longitudinal distribution of vascular resistance was studied using the arterial, venous, and double occlusion method (Dawson *et al.* 1987). The occlusions were carried out under the same conditions as the pressure/flow measurement. The pressure, which was measured just downstream from the site of sudden occlusion of the inflow tubing by an electromagnetically operated clamp, decreased in time. The decrease followed two distinct exponentials: fast and slow. The transition between them was found by a manual graphic extrapolation of both exponentials and was taken to represent arterial occlusion pressure. The pressure drop between inflow and arterial occlusion pressures was considered to result from the resistance of larger pulmonary arteries (Dawson *et al.* 1987).

The simultaneous occlusion of both the inflow and outflow tubing, necessary for a successful measurement of the double occlusion pressure, was achieved by using two electromagnetic clamps controlled by one common switch. After the double occlusion maneuver, the pressure measured just downstream from the inflow clamp fell and the pressure measured just upstream from the outflow clamp rose. Both pressures met at a common value considered to be mean capillary pressure (Dawson *et al.* 1987). The pressure drop between arterial and double occlusion can be considered to correspond to the resistance in the arterial side of the microcirculation.

The pressure measured just upstream from the site of an instant, manual occlusion of the venous outflow rose in two phases: the first was very fast and this was followed by a slower phase. The transition between the two phases was easy to detect and was considered to be the venous occlusion pressure. The pressure drop between the double and venous occlusion pressures reflects the resistance of the post-capillary side of the microcirculation. The difference between the venous occlusion and outflow pressures corresponds to the resistance of larger veins (Dawson *et al.* 1987).

Dose-response to angiotensin II

The relationship between the dose of angiotensin II and the magnitude of the vasoconstrictor response was measured using a series of bolus injections into the inflow line containing 0.1, 0.2, 0.3, 0.4 and 0.5 μg of angiotensin II. Each bolus caused a transient increase in perfusion pressure, and the difference between the pressure before and at the peak of the response was taken as the magnitude of the response.

Statistical evaluation

Data are presented as means ± S.E.M. Two group comparisons were performed using Student's unpaired t-test. Factorial analysis of variance (ANOVA) was used whenever three groups were compared. The pressure/flow curves and the dose response curves (angiotensin II) were analyzed using repeated measurements ANOVA. $P<0.05$ was considered significant.

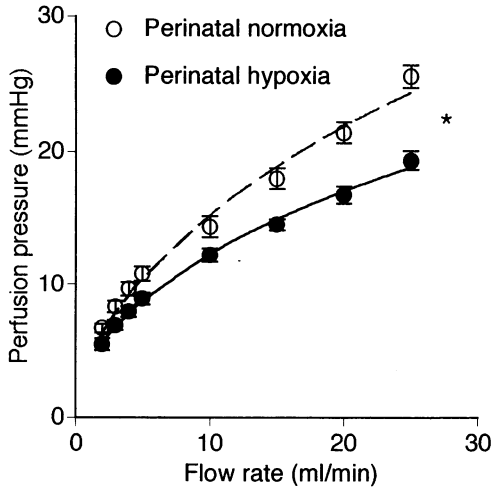

Fig. 3. *Perinatal experience of hypoxia tilts the perfusion pressure/flow relationship in lungs isolated from adult rats towards lower pressures. The data were obtained under zone 3 conditions and analyzed by non-linear regression. Individual values are group means ± S.E.M., curves are non-linear regressions for each group. n = 8 per group. * The difference between the pressure/flow curves is significant (P < 0.0001 by repeated measures ANOVA).*

Results

At the time of sacrifice there were no significant differences in body weight among the groups: perinatally normoxic controls 332±7 g, perinatally hypoxic rats 389±23 g, rats in the supplementary experiment 352±50 g ($P = 0.26$).

Pressure/flow relationships

Baseline perfusion pressures in the isolated lungs were slightly lower in the adult rats born in hypoxia than in the controls (13.8±0.4 vs. 15.5±0.6 mm Hg, $P = 0.03$). As in our previous experiments (Herget and Hampl 1990, Herget and Kuklík 1995), the pressure/flow curves were shifted towards the flow axis in the perinatally hypoxic as compared to the control rats (Fig. 3). All data fit well to the non-linear model of Linehan *et al.* (1992).

The results of the non-linear regression analysis of the pressure/flow data are shown in Figure 4. The parameter α (corresponding to vascular distensibility) was significantly elevated by the perinatal experience of hypoxia, while the parameter R_0 was unaffected.

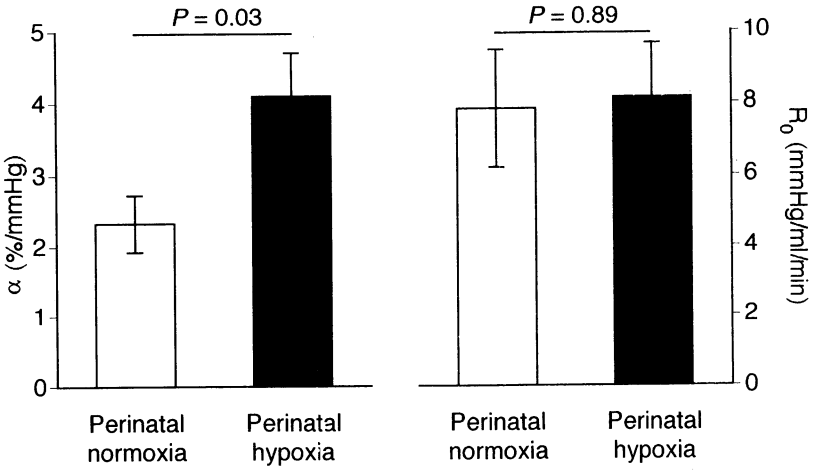

Fig. 4. *Results of the non-linear regression analysis of the pressure/flow data. Perinatal hypoxia increases the parameter α (corresponding to vascular distensibility, left) and does not alter the parameter R_0 (~resistance at near-zero flow, right). n = 8 per group.*

In the supplementary experiment, where the rats were exposed to hypoxia when mature and then allowed to recover in room air for ~12 weeks, the pressure/flow curves did not differ significantly from the controls (data not shown). In fact, there was a non-significant tendency towards higher pressures (rather than lower pressures as in the perinatally hypoxic group). Both parameters of the non-linear regression analysis did not differ significantly from the control group (α: 2.5±0.4 vs. 2.3±0.4 %/mm Hg, P=0.80; R_0: 11.7±2.8 vs. 7.8±1.6 mm Hg/ml/min, P=0.23).

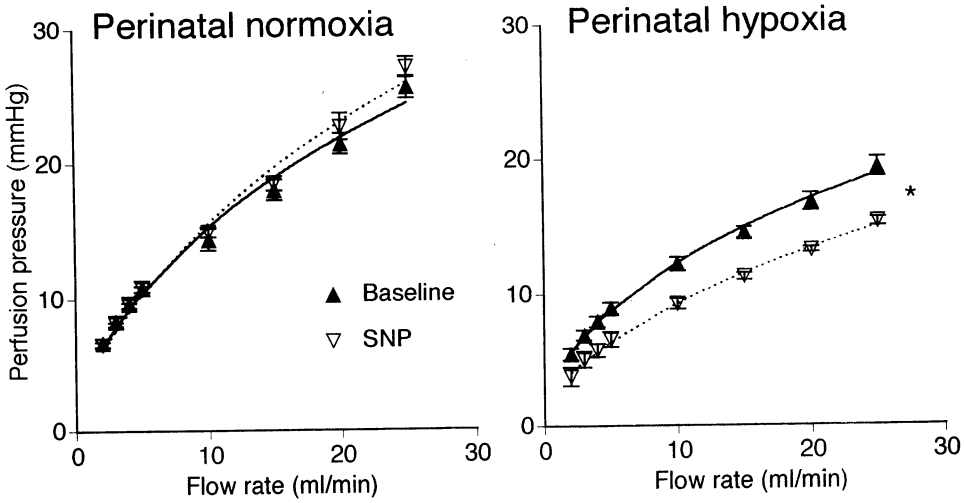

Fig. 5. *Elimination of vascular tone by sodium nitroprusside shifts the pressure/flow curves towards lower pressures only in the lungs of perinatally hypoxic rats. The data were obtained under zone 3 conditions and analyzed by non-linear regression. Individual values are group means ± S.E.M., curves are non-linear regressions for each group. * The effect of sodium nitroprusside in the perinatally hypoxic group is significant (P = 0.007 by repeated measures ANOVA).*

Sodium nitroprusside

In the perinatally normoxic group, the pressure/flow curves were unaffected by sodium nitroprusside administration (Fig. 5, left). This finding is consistent with a virtual absence of basal tone in the pulmonary vessels, well established previously by others (Emery *et al.* 1981, Adnot *et al.* 1988, Hyman and Kadowitz 1988). In the perinatally hypoxic group, sodium nitroprusside shifted the pressure/flow curves to lower pressures ($P<0.007$, Fig. 5, right). This implies that, after the perinatal experience of hypoxia, basal tone of the vascular smooth muscle cells contributed significantly to the resting resistive properties of the pulmonary circulation.

The downward shift in the pressure/flow curves after sodium nitroprusside in the perinatally hypoxic group was predominantly due to a decrease in R_0. This parameter did not differ in the groups before sodium nitroprusside administration (Fig. 4), but was significantly lower in the perinatally hypoxic (4.1±0.5 mm Hg/ml/min) than in the normoxic (6.4±0.4 mm Hg/ml/min; P=0.014) group after sodium nitroprusside. The parameter α did not change with sodium nitroprusside in either group and remained significantly higher in the perinatally hypoxic than normoxic group (3.4±0.7 vs. 1.7±0.2 %/mm Hg; P=0.01).

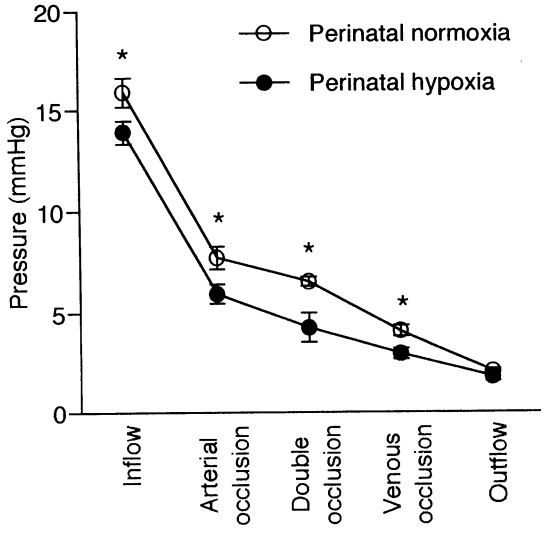

Fig. 6. *Inflow pressure and all occlusion pressures are lower in lungs of adult rats with the perinatal experience of hypoxia. n = 8 per group. *P < 0.05*

Occlusions

The occlusion data are summarized in Figure 6. The inflow pressure, arterial occlusion pressure, double occlusion pressure, and venous occlusion pressure were all significantly lower in rats born in hypoxia than in rats born in normoxia.

In the supplementary experiment, the perinatally normoxic rats exposed to hypoxia as adults and then recovering in room air for 3 months had inflow and occlusion pressures, which did not significantly differ from the values in perinatally normoxic controls (data not shown). In fact, the inflow and occlusion pressures tended to be higher in the supplementary group than in controls, although significance had not been reached.

Angiotensin II

Although the pressures at a given flow rate were lower in the lungs of perinatally hypoxic than in normoxic rats (Fig. 3), it is possible that this may be offset *in vivo* by an increased reactivity to blood borne vasoconstrictors. To test this possibility, we chose a potent pulmonary vasoconstrictor normally present in the blood, angiotensin II, and measured the dose-response relationship. We found that the dose-response curve was shifted significantly downwards in the lungs of the perinatally hypoxic as compared to perinatally normoxic rats (Fig. 7).

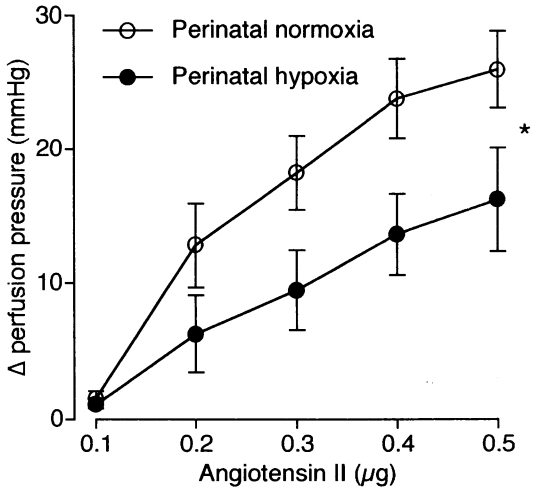

Fig. 7. *Dose response to angiotensin II is shifted towards lower pressure responses in lungs from adult rats with the perinatal history of hypoxia. n = 5 per /group. * The downward shift of the dose-response curve is significant (P < 0.05 by repeated measures ANOVA).*

Discussion

The present data show that pulmonary perfusion pressures are lower over a wide range of flow rates in adult rats that had been exposed to perinatal hypoxia, as compared to rats without such an experience (Fig. 3). Non-linear regression analysis of the pressure/flow data indicates that this finding is attributable to increased vascular distensibility (Fig. 4). The resistance of the non-distended vessels is not altered. This rejects the possibility that a reduction of the total cross-sectional area of the pulmonary circulation is responsible for the downward shift of the pressure/flow curves (Herget and Hampl 1990). The data obtained using vascular occlusion methods suggest that the lower pressures in the perinatally hypoxic group are due to lower resistance in both the pre-capillary and post-capillary segment of the pulmonary vascular bed (Fig. 6). Interestingly, the pulmonary perfusion pressures were lower in the perinatally hypoxic group despite the increased basal vascular smooth muscle tone in this group (Fig. 5). Finally, the vasoconstrictor reactivity to angiotensin II was lower in the lungs of adult rats with a history of perinatal hypoxia as compared to rats without this exposure (Fig. 7).

Taken together, the present results are consistent with the possibility that perinatal experience of hypoxia alters the pulmonary vasculature in a way that may be expected to render the individual less prone to the development of pulmonary hypertension in adulthood. This interpretation appears to be in conflict with our previous findings and those of others.

We have reported that perinatal hypoxia leads to a tendency towards an increase in pulmonary artery pressure (21.1±1.8 *vs.* 18.5±1.1 mm Hg) in adult male rats. Statistical significance, however, was not reached (Herget *et al.* 1987, Hampl and Herget 1990). Similarly, the tendency towards right ventricular hypertrophy did not reach significance. The wet weight ratio of the right ventricle to the left ventricle plus septum was 0.32±0.01 in perinatally hypoxic and 0.24±0.01 in perinatally normoxic adult male rats (Herget *et al.* 1987, Hampl and Herget 1990).

In a similar study, Hakim and Mortola (1990) found significant right ventricular hypertrophy and increased vascular resistance in isolated perfused lungs of adult rats that had been exposed to hypoxia during their first postnatal week. In the preliminary report of Barer *et al.* (1997), right ventricular hypertrophy and pulmonary hypertension induced by perinatal exposure of rats to hypoxia was statistically significant in adult female but not in male rats. There is also a preliminary report of a significant hypertrophy of both right and left ventricles in adult rats with a perinatal history of hypoxia (Jones *et al.* 1999). Recently, we found significant right ventricular hypertrophy in adult females born in hypoxia, although the males were not affected (Hampl *et al.* 2000). Tang *et al.* (2000) reported that a few days of hypoxia in the perinatal period increased the severity of pulmonary hypertension when the rats were re-exposed to hypoxia at two weeks of age.

In addition, we have reported previously that male rats born in hypoxia had increased pulmonary vasoconstrictor reactivity to acute hypoxia during recovery from a chronic hypoxic exposure in adulthood (Hampl and Herget 1990). Hakim and Mortola (1990) reported increased hypoxic pulmonary vasoconstriction in adult rats that had been exposed to neonatal hypoxia even without any re-exposure to chronic hypoxia in adulthood. While hypoxic pulmonary vasoconstriction normally occurs in small pre-capillary arteries, the proximal segment of the pulmonary vasculature is also significantly involved in this response of adult rats with a perinatal history of hypoxia (Herget and Kuklík 1995). These findings imply a greater susceptibility to factors expected to promote the development of pulmonary hypertension. How can this be reconciled with our present findings implying protective effects of perinatal hypoxia?

We suggest that the adverse and beneficial consequences of perinatal hypoxia may co-exist in parallel. Perinatal hypoxia is well known to delay many aspects of pulmonary vascular maturation (Haworth 1988, Haworth 1995, Evans *et al.* 1998, Stiebellehner *et al.* 1998). It is possible that such pro-hypertensive alterations elicit compensatory mechanisms aimed at offsetting their influence and achieving and maintaining normal properties of the pulmonary vascular bed. The increased vascular distensibility and reduced vasoconstrictor reactivity to angiotensin II found in the present study may serve as examples of such compensatory mechanisms. On the other hand, the elevated basal tone (inferred from the increased vasodilator response to sodium nitroprusside) may represent a remnant of the pro-hypertensive influence of perinatal hypoxia. The actual state of the pulmonary circulation in adults with a perinatal experience of hypoxia then results from the balance between the beneficial and adverse consequences of perinatal hypoxia. It is likely that additional influences, such as changes in

hormonal status (Hampl *et al.* 2000) or re-exposure to hypoxia in adulthood (Hampl and Herget 1990) may tip this balance towards a greater susceptibility to the development of pulmonary vascular disease.

Acknowledgements

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References

ADNOT S, CHABRIER PE, BRUN-BUISSON C, VIOSSAT I, BRAQUET P: Atrial natriuretic factor attenuates the pulmonary pressor response to hypoxia. *J Appl Physiol* **65**: 1975-1983, 1988.

BARER G, EMERY C, LIU X, LAUDE E: Haemodynamic, metabolic and growth consequences of sustained hypoxia in mature and perinatal rats, studied in isolated perfused lungs. *J Physiol* **499P**: S10, 1997.

DAWSON CA, LINEHAN JH, BRONIKOWSKI TA, RICKABY DA: Pulmonary microvascular hemodynamics: occlusion methods. In: *The Pulmonary Circulation in Health and Disease.* JA WILL, CA DAWSON, EK WEIR, CK BUCKNER (eds), Academic Press, Orlando, 1987, pp 175-197.

EMERY CJ, BEE D, BARER GR: Mechanical properties and reactivity of vessels in isolated perfused lungs of chronically hypoxic rats. *Clin Sci* **61**: 569-580, 1981.

EVANS AM, OSIPENKO ON, HAWORTH SG, GURNEY AM: Resting potentials and potassium currents during development of pulmonary artery smooth muscle cells. *Am J Physiol* **275**: H887-H899, 1998.

FUNG Y-C: Pressure, flow, stress, and remodeling in the pulmonary vasculature. In: *The Pulmonary Circulation and Gas Exchange.* WW WAGNER, EK WEIR (eds), Futura, Armonk, New York, 1994, pp 343-364.

HAKIM TS, MORTOLA JP: Pulmonary vascular resistance in adult rats exposed to hypoxia in the neonatal period. *Can J Physiol Pharmacol* **68**: 419-424, 1990.

HAMPL V, HERGET J: Perinatal hypoxia increases hypoxic pulmonary vasoconstriction in adult rats recovering from chronic exposure to hypoxia. *Am Rev Respir Dis* **142**: 612-624, 1990.

HAMPL V, ARCHER SL, NELSON DP, WEIR EK: Chronic EDRF inhibition and hypoxia: effects on pulmonary circulation and systemic blood pressure. *J Appl Physiol* **75**: 1748-1757, 1993.

HAMPL V, TRISTANI-FIROUZI M, NELSON DP, ARCHER SL: Chronic infusion of nitric oxide in experimental pulmonary hypertension: pulmonary pressure-flow analysis. *Eur Respir J* **9**: 1475-1481, 1996.

HAMPL V, BÍBOVÁ J, HERGET J: Long-term effects of perinatal hypoxia on the pulmonary circulation: are they protective? *Physiol Res* **48**: 56 P, 1999.

HAMPL V, BÍBOVÁ J, OŠŤÁDALOVÁ I, HERGET J: Ovaries protect adult female rats born in hypoxia from the development of pulmonary hypertension (abstract). *FASEB J* **14**: A125, 2000.

HAWORTH SG: Pulmonary vascular remodeling in neonatal pulmonary hypertension: state of the art. *Chest* **93**: 133S-138S, 1988.

HAWORTH SG: Development of the normal and hypertensive pulmonary vasculature. *Exp Physiol* **80**: 843-853, 1995.

HERGET J, HAMPL V: Pulmonary vasculature of adult rats is influenced by perinatal experience of hypoxia. In: *Pulmonary Blood Vessels in Lung Disease.* J WIDIMSKÝ, J HERGET (eds), Karger, Basel, 1990, pp. 70-76.

HERGET J, KUKLÍK V: Perinatal lung injury extends in adults the site of hypoxic pulmonary vasoconstriction upstream. *Physiol Res.* **44**: 25-30, 1995.

HERGET J, HAMPL V, PALEČEK F: Effect of perinatal hypoxia on hypoxic vascular reactivity in adult rats. *Atemwegs- und Lungenkrankheiten* **13**: 132, 1987.

HYMAN AL, KADOWITZ PJ: Tone-dependent responses to acetylcholine in the feline pulmonary vascular bed. *J Appl Physiol* **64**: 2002-2009, 1988.

JONES RD, STEEDS RP, EMERY CJ, CHANNER KS, MORICE AH: Perinatal exposure to hypoxia affects the development of the pulmonary and coronary circulation of the adult rat. *Physiol Res* **48**: 56P, 1999.

LINEHAN JH, HAWORTH ST, NELIN LD, KRENZ GS, DAWSON CA: A simple distensible vessel model for interpreting pulmonary vascular pressure-flow curves. *J Appl Physiol* **73**: 987-994, 1992.

MITZNER W, HUANG I: Interpretation of pressure-flow curves in the pulmonary vascular bed. In: *The Pulmonary Circulation in Health and Disease.* JA WILL, CA DAWSON, EK WEIR, CK BUCKNER (eds), Academic Press, Orlando, 1987, pp 215-230.

STIEBELLEHNER L, BELKNAP JK, ENSLEY B, TUCKER A, ORTON EC, REEVES JT, STENMARK KR: Lung endothelial cell proliferation in normal and pulmonary hypertensive neonatal calves. *Am J Physiol* **275**: L593-L600, 1998.

TANG J-R, CRAS TDL, MORRIS KG, ABMAN SH: Brief perinatal hypoxia increases severity of pulmonary hypertension after reexposure to hypoxia in infant rats. *Am J Physiol* **278**: L356-L364, 2000.

Reprint requests

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