

Effects of Perinatal Exposure to Hypoxia upon the Pulmonary Circulation of the Adult Rat

R. D. JONES, A. H. MORICE, C. J. EMERY¹

Section of Respiratory Medicine, Division of Academic Medicine, University of Hull, Castle Hill Hospital, Cottingham, ¹Respiratory Medicine, Division of Clinical Sciences (South), University of Sheffield, Sheffield, United Kingdom

Received January 7, 2003

Accepted May 12, 2003

Summary

The hypothesis on *Fetal and Infant Origins of Adult Disease* proposes that an altered *in utero* environment may impair fetal development and physiological function, increasing susceptibility to disease in adulthood. Previous studies demonstrated that reduced fetal growth predisposes to adult cardiovascular diseases. Maternal smoking and high altitude are also linked to reduced fetal growth and adult disease, and both cause fetal hypoxia. We therefore wanted to determine whether fetal hypoxia produces alterations in the adult pulmonary vasculature. Body and ventricular weight, pulmonary arterial compliance and vasoreactivity to potassium chloride (KCl), prostaglandin F_{2α} (PGF_{2α}), acetylcholine (ACh) and sodium nitroprusside (SNP) were studied in adult rats exposed to 10 % hypoxia throughout the perinatal period, compared to age-matched controls. Rats exposed to perinatal hypoxia had reduced body weight (199±15 vs. 294±10 g, P<0.001), elevated right ventricular weight (70.3±8.8 vs. 51.4±1.2 mg/100 g, P<0.05), elevated left ventricular weight (281±27 vs. 232±5 mg/100 g, P<0.05), reduced pulmonary arterial compliance (35.2±2.0 vs. 46.4±2.4 μm/mN, P<0.05) and reduced maximal pulmonary vasoconstriction to KCl (1.74±0.14 vs. 2.63±0.31 mN/mm, P<0.01), and PGF_{2α} (1.40±0.14 vs. 2.47±0.44 mN/mm, P<0.05). Perinatal exposure to hypoxia had a profound effect upon the adult pulmonary circulation, which could predispose to cardiopulmonary diseases in adulthood.

Key words

Perinatal hypoxia • Vascular reactivity • Vascular compliance • Vascular remodelling

Introduction

The hypothesis on *Fetal and Infant Origins of Adult Disease* (Barker 1993) highlights the link between fetal undernutrition, and the occurrence of diseases of the cardiovascular system in adult life. Markers of fetal undernutrition such as low birth weight, low birth weight/placental weight ratio, small head circumference at birth, and thinness at birth are all associated with increased death rates from cardiovascular diseases in

adult humans (Barker *et al.* 1989, 1993a,b). A reduction in the rate of fetal growth is also associated with subsequent development of coronary heart disease in later life (Barker 1995). Evidence from animal models supports this link. Offspring of rats fed a low protein diet develop significantly higher systolic blood pressure which persists to maturity, compared to offspring of dams fed on a normal diet (Langley and Jackson 1994). The hypothesis proposes that any alterations to the fetal environment, which could potentially interfere with fetal

development, may irreversibly impair physiological function, increasing susceptibility to disease in later life (Barker 1994).

It is widely accepted that exposure to adverse conditions *in utero* can affect the development of the fetus. Smoking during pregnancy is probably the most documented example, being associated with reduced infant birth weight (Secker-Walker *et al.* 1995, Wald and Hackshaw 1996) and a reduction in the rate of fetal growth (Olsen 1992, Koren 1995). Whilst the toxicity of substances present in cigarette smoke such as nicotine and carbon monoxide, could be the predominant cause of these effects, it is conceivable that fetal hypoxia, which is also a consequence of smoking (Cnattingius and Nordstrom 1996), could be involved. Fetal hypoxia is also triggered by both nicotine, which causes uterine and umbilical artery contraction, increasing maternal blood pressure and reducing uterine blood flow, and also carbon monoxide, which combines with hemoglobin to form carboxyhemoglobin and inhibits the release of oxygen into fetal tissues (Lambers and Clark 1996). Indeed, fetal hypoxia is also associated with low birth weight (Witlin 1997) and reduced fetal growth (Lueder *et al.* 1995).

Evidence also suggests that hypoxia at high altitude results in reduced neonatal oxygenation, which in turn is associated with uterine growth retardation and less complete neonatal cardiopulmonary transition. Such effects have a lower incidence in lifelong residents at high altitude, compared to acclimatized newcomers (Moore 1987). In addition, increased maternal ventilation at high altitude is positively correlated with arterial oxygenation and infant birth weight (Moore *et al.* 1986) and reduced oxygen availability at high altitude is associated with increased infant mortality (Sui *et al.* 1988).

Consequently, fetal hypoxia could also predispose to cardiovascular disease in adulthood, a hypothesis which has yet to be investigated directly. We have studied the effects of perinatal hypoxia upon body weight, left and right ventricular hypertrophy, and pulmonary artery compliance and vasoreactivity in the adult rat. The objective of the study was to determine whether these parameters, which act as markers for cardiovascular disease, and in particular pulmonary hypertension, are affected by fetal and neonatal hypoxia.

Methods

Perinatal animal treatment

This investigation conforms to the *Animals (Scientific Procedures) Act 1986* concerning the welfare

of laboratory animals, and was undertaken under the jurisdiction of a UK Home Office Project License. Pregnant Wistar dams were exposed to hypoxia (10 % oxygen) in a normobaric chamber for 1 week *pre* and 1 week *post partum*. Intervention began on the 14th day of gestation – the onset of the perinatal phase. Dams received an unlimited supply of food and tap water and were supervised on a daily basis. Pups were weaned as normal and the males sacrificed at adulthood (between 8 and 10 weeks of age) (PNCH). Age-matched males from untreated dams were used as controls (PNC).

Vessel preparation

The adult male offspring (n=11) and controls (n=9) were anesthetized between 56–65 days of age by intraperitoneal injection of sodium pentobarbitone (50 mg/kg) and then killed by cervical dislocation. The heart and lungs were removed and placed in chilled physiological saline solution (PSS). Pulmonary arteries (n=33, mean internal diameter 637 ± 33 μm) were dissected, mounted in a wire myograph and loaded to resting tension equivalent to the normal *in vivo* pressure of 17.5 mm Hg (Rogers *et al.* 1992). The myograph bath was then filled with 5ml PSS heated to 37 °C and bubbled with 95 % O₂ / 5 % CO₂. The vessels were allowed to equilibrate for 1 hour.

Animal growth

Measurements of body weight, and right and left ventricular weight per 100 g body weight were made in PNCH and control animals.

Vessel compliance

Dynamic diameter-tension curves were obtained for selected pulmonary arteries (n=10), following mounting in the myograph. Starting from a diameter which yielded minimal tension, each vessel was mechanically stretched *via* separation of the myograph jaws at a rate of 50 $\mu\text{m/s}$, until the force exerted upon it reached a maximum tension of 25 mN. The myograph jaws were then returned to their original position at the same rate until zero tension was obtained. The x/y value of the diameter-tension curve was calculated over the diameter range 600–800 μm , as a measure of dynamic vessel compliance (as $\mu\text{m/mN}$). These diameter ranges was chosen as they encompassed the resting diameters of the vessels studied, and were therefore representative of the *in vivo* variability in diameter.

Vessel reactivity

Initially the baseline tension of every vessel was

recorded and a contraction to a maximal concentration of potassium chloride (80 mM KCl) was measured. The vessels were then washed with fresh PSS and allowed to relax, until a stable baseline was regained. This maximal contractile response to KCl was then repeated to ensure reproducibility of the contractile response within each vessel. Following these preliminary experiments the test contractions were obtained. Pulmonary arteries were exposed to cumulative concentrations of two vasoconstrictors; prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and KCl, and two vasodilators; acetylcholine (ACh) and sodium nitroprusside (SNP).

A cumulative concentration-response curve to $PGF_{2\alpha}$ (1-100 μ M) was initially constructed and once a maximal response had been reached, ACh (0.1-100 μ M) was added cumulatively. Responses to ACh were expressed as a percentage relaxation of the $PGF_{2\alpha}$ precontraction. The vessels were then washed with fresh PSS and the original baseline tension regained. Following a 15 min equilibration period, a cumulative concentration-response curve to KCl (1-100 mM) was produced before the vessels were again washed with fresh PSS and the original baseline tension regained. A maximal concentration of $PGF_{2\alpha}$ (100 μ M) was then produced following another 15 min equilibration period, and once the vessels had maximally contracted, SNP (0.2 nM-20 μ M) was added cumulatively. Responses to SNP were expressed as a percentage relaxation of the $PGF_{2\alpha}$ precontraction.

Values of E_{max} (the maximal response produced), and $-\log EC_{50}$ (the negative \log_{10} of the concentration needed to produce 50 % of the maximal response), were obtained from the concentration-response curves as a measure of agonist efficacy and potency, respectively.

Solutions and drugs

PSS consisted of 120 mM NaCl, 4.7 mM KCl, 1.17 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.18 mM KH_2PO_4 , 5.5 mM glucose, 2.5 mM $CaCl_2$ and 26.9 μ M EDTA dissolved in distilled water.

All experimental reagents were obtained from Sigma (Poole, UK) and were dissolved in distilled water to the required concentration.

Statistical analysis

Values are expressed as means \pm S.E.M. and were compared using the Student's unpaired t test or Mann-Whitney U test, where appropriate. Significance was assumed with values of $P < 0.05$.

Results

Body weights

The body weights of the rats exposed perinatally to hypoxia were significantly smaller than age-matched controls. Adult PNCH offspring weighed 199 ± 15 g at 8-9 weeks compared to 294 ± 10 g of PNC controls ($P < 0.001$, Student's unpaired t test).

Ventricular weights

Relative weights of right ventricle (RV/100 g b.w.) and left ventricle (LV/100 g b.w.) were both significantly higher in PNCH animals. RV/100g was 70.3 ± 8.8 mg in PNCH animals compared to 51.4 ± 1.2 mg in PNC controls ($P < 0.05$). LV/100g was 281 ± 27 mg in PNCH animals compared to 232 ± 5 mg in PNC controls ($P < 0.05$).

Vessel compliance

Pulmonary arteries from rats exposed to perinatal hypoxia had a significantly lower compliance compared to vessels of a similar diameter from age-matched controls. Pulmonary arteries from PNC animals ($n=5$) had a dynamic vessel compliance of 45.4 ± 2.4 mm/mN which was reduced to 35.2 ± 2.0 mm/mN in vessels ($n=5$) from PNCH animals ($P < 0.05$).

Vessel reactivity

No significant differences were seen in the size of vessels selected for investigation in this study. Pulmonary arteries from PNCH animals ($n=18$) had a mean internal diameter of 606 ± 45 μ m compared to 673 ± 50 μ m of vessels from PNC controls ($n=15$) ($P > 0.1$).

Perinatal exposure to hypoxia resulted in a significant reduction in pulmonary artery reactivity to the vasoconstrictors $PGF_{2\alpha}$ and KCl compared to control vessels (Fig. 1). A significant reduction in vasoconstrictor efficacy to PGF was observed at all concentrations tested (1-100 μ M) and to KCl (at 30, 80 and 100 mM): E_{max} (KCl) was 1.74 ± 0.14 mN/mm in pulmonary arteries from PNCH animals compared to 2.63 ± 0.31 mN/mm in vessels from PNC controls ($P < 0.01$). Similarly E_{max} ($PGF_{2\alpha}$) was 1.40 ± 0.14 mN/mm in pulmonary arteries from PNCH animals compared to 2.47 ± 0.44 mN/mm in vessels from PNC controls ($P < 0.05$). However, no significant effect was seen on the $-\log EC_{50}$ values of these vasoconstrictive agonists, indicating no effect upon agonist potency: $-\log EC_{50}$ (KCl) was 1.85 ± 0.08 in pulmonary arteries from PNCH animals compared to 1.88 ± 0.04 in vessels from PNC controls, whilst $-\log EC_{50}$ ($PGF_{2\alpha}$) was

5.35±0.06 in pulmonary arteries from PNCH animals compared to 5.36±0.08 in vessels from PNC controls (both $P>0.1$).

Perinatal exposure to hypoxia had no significant effect upon vasoreactivity to either ACh or SNP (Fig. 2). E_{max} and $-\log EC_{50}$ values to both dilators were similar in vessels from PNCH and PNC animals, indicating no change in agonist efficacy or potency: E_{max} (ACh) was $-69.9\pm 5.4\%$ in pulmonary arteries from PNCH animals compared to $-73.3\pm 5.0\%$ in vessels from PNC controls,

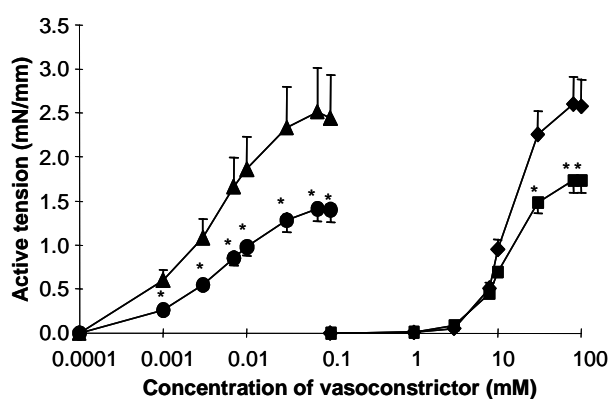


Fig. 1. Concentration-response curves for $PGF_{2\alpha}$ and KCl in isolated pulmonary arteries from rats exposed perinatally to 10% hypoxia (PNCH) compared to age-matched controls (PNC). Triangles – $PGF_{2\alpha}$ in PNC vessels; circles – $PGF_{2\alpha}$ in PNCH vessels; diamonds – KCl in PNC vessels; squares – KCl in PNCH vessels. All values are means \pm S.E.M.; * represents significance of $P<0.05$ by Student's unpaired t test.

Discussion

This work provides evidence that perinatal exposure to chronic hypoxia has profound consequences for the pulmonary circulation of the adult rat. Perinatal exposure to hypoxia resulted in a reduced body weight, increased right and left ventricular weight indicative of right and left ventricular hypertrophy, reduced pulmonary arterial compliance and alterations in pulmonary artery reactivity. Reduced pulmonary arterial compliance and right ventricular hypertrophy are markers of pulmonary remodeling associated with pulmonary hypertension, whilst left ventricular hypertrophy is indicative of the development of systemic hypertension. Reductions in pulmonary arterial reactivity could also have consequences for development of cardiovascular disease: The pulmonary circulation needs to be able to respond correctly to vasoactive stimuli. Pulmonary vasoconstriction in response to alveolar hypoxia or

whilst E_{max} (SNP) was $-75.3\pm 4.8\%$ in pulmonary arteries from PNCH animals compared to $-73.5\pm 6.2\%$ in vessels from PNC controls (both $P>0.1$, Mann-Whitney U test). Similarly, $-\log EC_{50}$ (ACh) was 6.29 ± 0.13 in pulmonary arteries from PNCH animals compared to 6.06 ± 0.12 in vessels from PNC controls, whilst $-\log EC_{50}$ (SNP) was 7.20 ± 0.35 in pulmonary arteries from PNCH animals compared to 7.12 ± 0.11 in vessels from PNC controls (both $P>0.1$, Mann-Whitney U test).

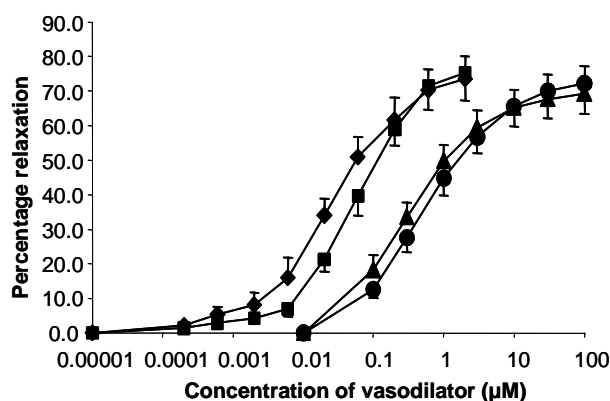


Fig. 2. Concentration-response curves for SNP and ACh in isolated pulmonary arteries from rats exposed perinatally to 10% hypoxia (PNCH) compared to age-matched controls (PNC). Diamonds – SNP in PNC vessels; squares – SNP in PNCH vessels, triangles – ACh in PNC vessels, circles – ACh in PNCH vessels. All values are means \pm S.E.M.

inflammatory mitogens, for example, ensures that blood is diverted from underventilated areas of the lung or regions of local infection. Clearly inappropriate or impaired vasoconstrictive responses under such conditions may result in an increased susceptibility to pulmonary disease.

Chronic hypoxia is well known to elicit remodeling of the pulmonary circulation (Hunter *et al.* 1974, Emery *et al.* 1981), but few studies have demonstrated changes induced by perinatal exposure to hypoxia. The fetus is well protected from hypoxia *via* fetal hemoglobin, which has a much higher affinity for oxygen compared to the adult form. Nevertheless, in adult rats which had received perinatal exposure to the same degree of hypoxia as in the present study, there are alterations of perfusion pressure/flow relationships (Herget and Hampl 1990, Hampl *et al.* 2000), attributed to a permanent increase in the pulmonary basal tone (Hampl *et al.* 2000). Such observations confer with the

findings of the present study, where we have shown that perinatal exposure to chronic hypoxia results in a reduction of pulmonary arterial compliance in conjunction with an increase of right ventricular weight in adulthood. The observed reduction in vessel compliance indicates that these vessels have undergone some degree of vascular remodelling, consistent with the development of pulmonary hypertension. Evidence of remodeling is also provided by the observed increase in right ventricular weight, indicative of right ventricular hypertrophy analogous to pulmonary hypertension. However, in contrast, previous work failed to demonstrate an increase in the percentage of thick walled peripheral pulmonary vessels in adult rats treated similarly to those in the present study (Hampl and Herget 1990). We have also demonstrated that exposure to hypoxia for 1 week *pre* and 1 week *post partum* produces a marked reduction in pulmonary artery reactivity to the vasoconstrictors $\text{PGF}_{2\alpha}$ and KCl. The relaxant ability of the vessels, as indicated by responsiveness to ACh and SNP, was unaffected. This reduction in contractile activity may be a consequence of the vascular remodeling observed in these vessels. In contrast, Hampl and Herget (1990) reported no difference in the vasoconstrictive response to hypoxia in isolated perfused lungs obtained from adult rats exposed perinatally to hypoxia. However, the differences between the results of the present and this previous study (Hampl and Herget 1990), both in terms of remodeling and vasoreactivity, may be due to the fact that the study was made in different portions of the pulmonary vasculature. Alternatively the differing vasoconstrictive mechanisms activated following exposure to $\text{PGF}_{2\alpha}$, KCl and acute hypoxia may be responsible.

The vascular changes observed in the present study following perinatal exposure to hypoxia, are consistent with those reported following exposure to chronic hypoxia in adulthood, which ultimately results in the development of pulmonary hypertension and vascular remodelling (Hunter *et al.* 1974, Emery *et al.* 1981). Maximal response to KCl (Youngson *et al.* 1993) and hypoxic pulmonary vasoconstriction (HPV) (McMurtry *et al.* 1978, Youngson *et al.* 1993) have previously been shown to be reduced in isolated vessel and lung preparations from rats exposed to chronic hypoxia. Similarly, no significant differences have been shown between the responses to SNP and ACh of pulmonary arteries from rats exposed to chronic hypoxia compared with the controls (Teng 1995), although in the same study there was also no significant difference in the response to

$\text{PGF}_{2\alpha}$. However, in contrast, other studies reported that exposure to chronic hypoxia was associated with an increase in agonist-induced pulmonary vasoconstriction (Rodman 1992, Maruyama and Maruyama 1994), although these two studies utilized much smaller vessels (150-300 μm). Clearly the issue of vessel size is important, and may explain the differences between this and previous studies. The composition of the rat pulmonary vasculature varies markedly throughout the vascular bed, with an increase in smooth muscle cell content observed in smaller, resistance arteries (Sasaki *et al.* 1995). The pulmonary arteries utilized in the present study came from the transitional elastic segment, compared to the resistance arteries from the thick muscular segment used in other studies (Rodman 1992, Maruyama and Maruyama 1994, Hampl and Herget 1990, Sasaki *et al.* 1995). As well as differences in their composition, smaller vessels are also recognized to exhibit enhanced vasoconstrictor responses (Leach *et al.* 1992).

Clearly, similarities exist between the effects of perinatal and adult exposure to chronic hypoxia upon the pulmonary circulation. We have demonstrated that exposure to hypoxia during the perinatal phase has affected the normal development of the fetal lung, resulting in significant structural and functional alterations to the adult pulmonary circulation. It would appear that perinatal exposure to chronic hypoxia produces remodeling similar to that seen following chronic exposure to hypoxia in adulthood, potentially leading to development of pulmonary hypertension. In addition, perinatal exposure to chronic hypoxia also reduced normal body growth and has consequences for the systemic circulation since evidence of left ventricular hypertrophy, an indicator of hypertensive systemic remodeling, was obtained. However, the perinatal period covers the interval spanning from one week *pre* to one week *post partum*, and the animals utilized in the present study were exposed to hypoxia for the duration of this period. Consequently, it is not possible to determine at this stage, whether exposure to hypoxia over the entire perinatal period or just the prenatal period, the postnatal period, or at the time of birth, is associated with the described structural and functional alterations. Indeed, a reduced body weight and hypoplasia of the lungs is evident in newborn mammals following exposure to hypoxia for several days immediately following birth (Cunningham *et al.* 1974, Teitel *et al.* 1985, Mortola *et al.* 1986, Massaro *et al.* 1989, Mortola *et al.* 1990). Similarly, the pulmonary circulation undergoes its most

dramatic functional change at birth, being transformed from a low flow/high resistance system to a high flow/low resistance system. This occurs *via* marked vasodilation, triggered by the increased oxygen concentration of the circulating blood which occurs once the lung is first ventilated at birth. Exposure to hypoxia at these phases of development, as well as *in utero* exposure, could all be involved. Clearly further study in this area is warranted.

In summary, we have demonstrated that perinatal exposure to chronic hypoxia has profound effects upon normal cardiovascular development which persists into adulthood. Reduced body weight, right and left ventricular hypertrophy, reduced pulmonary arterial compliance and alterations in pulmonary artery contractility, which were observed in adulthood, were all consequences of perinatal exposure to chronic hypoxia. The '*Barker Hypothesis*' (Barker 1993) proposes that any

alteration of the *in utero* environment which could potentially interfere with fetal development may irreversibly impair physiological function, increasing susceptibility to disease in later life. Our findings support this hypothesis since right ventricular hypertrophy and reduced pulmonary arterial compliance are potential markers of pulmonary hypertension, left ventricular hypertrophy is consistent with systemic hypertension, and altered pulmonary arterial reactivity may also predispose to cardiovascular disease. Reduced growth has previously been linked to development of cardiovascular disease in later life. It would appear that avoidance of fetal hypoxia as well as fetal undernutrition is also essential for normal cardiovascular development.

Acknowledgements

R. D. Jones was supported by an ML Laboratories Ph.D. studentship.

References

- BARKER DJ: *Foetal and Infant Origins of Adult Disease*. British Medical Journal Press, London, 1993.
- BARKER DJ: *Mothers, Babies and Disease in Later Life*. British Medical Journal Press, London, 1994.
- BARKER DJ: Fetal origins of coronary heart disease. *Br Med J* **311**: 171-174, 1995.
- BARKER DJ, WINTER PD, OSMOND C, MARGETTS B, SIMMONDS SJ. Weight in infancy and death from ischaemic heart disease. *Lancet* **2**: 577-580, 1989.
- BARKER DJ, GLUCKMAN PD, GODFREY KM, HARDING JE, OWENS JA, ROBINSON JS: fetal nutrition and cardiovascular disease in adult life. *Lancet* **341**: 938-941, 1993a.
- BARKER DJ, OSMOND C, SIMMONDS SJ, WIELD GA. The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. *Br Med J* **306**: 422-426, 1993b.
- CNATTINGIUS S, NORDSTROM ML: Maternal smoking and foeto-infant mortality: biological pathways and public health significance. *Acta Paediatr* **85**: 1400-1402, 1996.
- CUNNINGHAM EL, BRODY JS, JAIN BP: Lung growth induced by hypoxia. *J Appl Physiol* **37**: 362-366, 1974.
- 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.
- HAMPL V, HERGET J: Perinatal hypoxia increases hypoxic pulmonary vasoconstriction in adult rats recovering from chronic exposure to hypoxia. *Am Rev Resp Dis* **142**: 619-624, 1990.
- HAMPL V, BÍBOVÁ J, HERGET J: Perinatal history of hypoxia leads to lower vascular pressures and hyporeactivity to angiotensin II in isolated lungs of adult rats. *Physiol Res* **49**: 567-575, 2000.
- HERGET J, HAMPL V: Pulmonary vasculature of adult rats is influenced by perinatal exposure of hypoxia. In: *Pulmonary Blood Vessels in Lung Disease*. J WIDIMSKÝ, J HERGET (eds), Karger, Basel, 1990, pp. 70-76.
- HUNTER C, BARER GR, SHAW JW, CLEGG EJ: Growth of the heart and lungs in hypoxic rodents: a model of human hypoxic disease. *Clin Sci Mol Med* **46**: 375-391, 1974.
- KOREN G: Fetal toxicology of environmental tobacco smoke. *Curr Opin Pediatr* **7**: 128-131, 1995.
- LAMBERS DS, CLARK KE: The maternal and fetal physiologic effects of nicotine. *Semin Perinatol* **20**: 115-126, 1996.
- LANGLEY SC, JACKSON AA: Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. *Clin Sci* **86**: 217-222, 1994.
- LEACH RM, TWORT CH, CAMERON IR, WARD JP: A comparison of the pharmacological and mechanical properties in vitro of large and small pulmonary arteries of the rat. *Clin Sci* **82**: 55-62, 1992.

- LUEDER FL, KIM SB, BUROKER CA, BANGALORE SA, OGATA ES: Chronic maternal hypoxia retards fetal growth and increases glucose utilisation of select fetal tissues in the rat. *Metabolism* **44**: 532-537, 1995.
- MARUYAMA J, MARUYAMA K: Impaired nitric oxide-dependent responses and their recovery in hypertensive pulmonary arteries of rats. *Am J Physiol* **266**: H2476-H2488, 1994.
- MASSARO GD, OLIVIER J, MASSARO D: Short-term perinatal 10% oxygen alters postnatal development of lung alveoli. *Am J Physiol* **257**: L221-L225, 1989.
- McMURTRY IF, PETRUN MD, REEVES JT: Lungs from chronically hypoxic rats have decreased pressor response to acute hypoxia. *Am J Physiol* **235**: H104-H109, 1978.
- MOORE LG: Altitude-aggravated illness: examples from pregnancy and prenatal life. *Ann Emerg Med* **16**: 965-973, 1987.
- MOORE LG, BRODEUR P, CHUMBE O, D'BROT J, HOFMEISTER S, MONGE C: Maternal hypoxic ventilatory response, ventilation and infant birth weight at 4,300 m. *J Appl Physiol* **60**: 1401-1406, 1986.
- MORTOLA JP, MORGAN CA, VIRGONA V: Respiratory adaptation to chronic hypoxia in newborn rats. *J Appl Physiol* **61**: 1329-1336, 1986.
- MORTOLA JP, XU LJ, LAUZON AM: Body growth, lung and heart weight, and DNA content in newborn rats exposed to different levels of chronic hypoxia. *Can J Physiol Pharmacol* **68**: 1590-1594, 1990.
- OLSEN J: Cigarette smoking in pregnancy and fetal growth. Does the type of tobacco play a role? *Int J Epidemiol* **21**: 279-284, 1992.
- RODMAN DM: Chronic hypoxia selectively augments rat pulmonary artery Ca²⁺ and K⁺ channel-mediated relaxation. *Am J Physiol* **263**: L88-L94, 1992.
- ROGERS TK, STEWART AG, MORICE AH: Effect of chronic hypoxia on rat pulmonary resistance vessels - vasodilatation by atrial natriuretic peptide. *Clin Sci* **83**: 723-729, 1992.
- SASAKI S, KOBAYASHI N, DAMBARA T, KIRA S, SAKAI T: Structural organisation of pulmonary arteries in the rat lung. *Anat Embryol* **191**: 477-489, 1995.
- SECKER-WALKER RH, SOLOMON LJ, FLYNN BS, SKELLY JM, LEPAGE SS, GOODWIN GD, MEAD PB: Smoking relapse prevention counselling during prenatal and early postnatal care. *Am J Prev Med* **11**: 86-93, 1995.
- SUI GJ, LIU YH, CHENG XS, ANAND IS, HARRIS E, HARRIS P, HEATH D: Subacute infantile mountain sickness. *J Pathol* **155**: 161-170, 1988.
- TEITEL D, SIDI D, BERNSTEIN D, HEYMANN MA, RUDOLPH AM: Chronic hypoxemia in the newborn lamb: cardiovascular, hematopoietic, and growth adaptations. *Pediatr Res* **19**: 1004-1010, 1985.
- TENG GQ: Altered reactivity of small pulmonary arteries of chronically hypoxic rats. In *Hypoxic Responses of Rat Pulmonary and Systemic Vessels in vitro*. GQ TENG (ed), The University of Sheffield, Sheffield, 1995, 72-90.
- WALD NJ, HACKSHAW AK: Cigarette smoking: an epidemiological overview. *Br Med Bull* **52**, 3-11, 1996.
- WITLIN AG: Asthma in pregnancy. *Semin Perinatol* **21**: 284-297, 1997.
- YOUNGSON C, NURSE C, YEGER H, CUTZ E: Oxygen sensing in airway chemoreceptors. *Nature* **365**: 153-155, 1993.

Reprint requests

Dr R.D. Jones, Academic Unit of Endocrinology, Division of Genomic Medicine, G Floor, The Medical School, Beech Hill Road, Sheffield, S10 2RX, UK, E-mail: R.D.Jones@sheffield.ac.uk