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Perindopril, an angiotensin converting enzyme inhibitor, in pulmonary hypertensive rats: comparative effects on pulmonary vascular structure and function

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1 Hypoxic pulmonary hypertension in rats (10% O_2, 4 weeks) is characterized by changes in pulmonary vascular structure and function. The effects of the angiotensin converting enzyme inhibitor perindopril (oral gavage, once daily for the 4 weeks of hypoxia) on these changes were examined.

2 Perindopril (30 mg kg^{-1} d^{-1}) caused an 18% reduction in pulmonary artery pressure in hypoxic rats.

3 Structural changes (remodelling) in hypoxic rats included increases in (i) critical closing pressure in isolated perfused lungs (remodelling of arteries <50 μm o.d.) and (ii) medial wall thickness of intralobar pulmonary arteries, assessed histologically (vessels 30–100 and 101–500 μm o.d.). Perindopril 10 and 30 mg kg^{-1} d^{-1} attenuated remodelling in vessels $\leqslant 100$ μm (lungs and histology), 30 mg kg^{-1} d^{-1} was effective in vessels 101–500 μm but neither dose prevented hypertrophy of main pulmonary artery. 3 mg kg^{-1} d^{-1} was without effect.

4 Perindopril (30 mg kg^{-1} d^{-1}) prevented the exaggerated hypoxic pulmonary vasoconstrictor response seen in perfused lungs from hypoxic rats but did not prevent any of the functional changes (i.e. the increased contractions to 5-HT, U46619 (thromboxane-mimetic) and K^+ and diminished contractions to angiotensins I and II) seen in isolated intralobar or main pulmonary arteries. Acetylcholine responses were unaltered in hypoxic rats.

5 We conclude that, in hypoxic rats, altered pulmonary vascular function is largely independent of remodelling. Hence any drug that affects only remodelling is unlikely to restore pulmonary vascular function to normal and, like perindopril, may have only a modest effect on pulmonary artery pressure.

Keywords: Pulmonary hypertension; perindopril; ACE inhibitor; pulmonary vascular remodelling; pulmonary vascular function; chronic hypoxia; pulmonary artery; isolated lungs

Abbreviations: ACE, angiotensin converting enzyme; 5-HT, 5-hydroxytryptamine

Introduction

In pulmonary hypertension the increase in pulmonary artery pressure is a consequence of at least two factors: (i) changes in pulmonary vascular structure (vascular remodelling), which include hypertrophy of the media and extension of smooth muscle into the normally non-muscular arteries in the alveolar region (Reid, 1979) and (ii) abnormal pulmonary vasoconstriction which reflects alterations in the functional properties of both the vascular smooth muscle and the endothelium (Dinh-Xuan, 1993; Gillespie *et al*., 1995). Current drug treatment of this disease involves the use of vasodilator drugs that oppose the abnormal vasoconstriction (Gaine & Rubin, 1998; Wanstall & Jeffery, 1998). In the future, an alternative approach may be to use drugs that attenuate or prevent vascular remodelling. One group of drugs that may have this property are the angiotensin converting enzyme (ACE) inhibitors since they prevent the formation of the potent smooth muscle mitogen, angiotensin II.

Captopril is the only ACE inhibitor that has been tested in humans with pulmonary hypertension and two out of the four long-term studies gave encouraging results (Leier *et al*., 1983; Pison *et al*., 1991; Alpert *et al*., 1992; Niazova *et al*., 1996). Captopril and also three of the more potent, long-acting ACE inhibitors, cilazapril, quinapril and ramipril, have been studied in rats with pulmonary hypertension (Clozel *et al*., 1991; Morrell *et al*., 1995b; Herget *et al*., 1996; Nong *et al*., 1996; Okada *et al*., 1998). Each of these drugs reduced pulmonary vascular remodelling, but to different degrees. Effects, when reported, on pulmonary artery pressure, right ventricular hypertrophy and systemic artery pressure, were variable, and no data were obtained on altered pulmonary vascular function. Perindopril is another potent, long-acting ACE inhibitor but it has not yet been studied in pulmonary hypertension. It belongs to the family of ACE inhibitors that contain a carboxyl group and is a prodrug for perindoprilat.

In humans the terminal elimination half-life of perindopril is >30 h and once daily administration is adequate (Salvetti, 1990). In rats, marked inhibition of ACE in lung tissue and pulmonary artery persists 24 h after a single oral dose of perindopril (1, 4 or 8 mg kg^{-1}), even though inhibition of plasma ACE can no longer be detected at this time (Jackson *et al*., 1988; Sakaguchi *et al*., 1988).

In this study we describe the effects of perindopril in rats that were exposed to hypoxia for 4 weeks to induce pulmonary hypertension. The specific aims were to examine the effects of perindopril on (i) pulmonary vascular remodelling and (ii)

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changes in pulmonary vascular function in this model of pulmonary hypertension. The studies on pulmonary vascular structure, which involved a range of doses of perindopril, utilized both a histological technique and also passive pressure–flow plots in isolated perfused lungs. None of the previous studies on ACE inhibitors has used both of these experimental approaches. The purpose of the functional studies was to assess whether a drug that prevents or attenuates vascular remodelling in pulmonary hypertension has the added benefit of restoring pulmonary vascular function to normal. Previous studies with ACE inhibitors, or with other potential anti-remodelling drugs, have not addressed this issue.

Preliminary accounts of these data have been communicated to the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (Jeffery & Wanstall, 1998a) and at the Second European Congress of Pharmacology (Jeffery & Wanstall, 1999).

Methods

Rats

Male Wistar rats, aged 8–9 weeks on the day of the experiment were used. Some of the rats were housed in hypoxic chambers (10% O_2) for 4 weeks before the experiment to induce pulmonary hypertension (Wanstall *et al*., 1992). Normoxic rats were housed in room air (21% O_2). Some of the rats in both the hypoxic and normoxic groups were treated with perindopril. Perindopril (dissolved in distilled water) was administered by oral gavage once a day throughout the 4 week exposure to hypoxia or normoxia. Perindopril doses used were 0.3, 3, 10 and 30 mg kg^{-1} d^{-1}.

Haemodynamic and heart weight measurements

In most rats systemic artery pressure and pulmonary artery pressure were determined on the day of the experiment as follows. Rats were anaesthetized with pentobarbitone (70 mg kg^{-1} i.p.) and were given an i.p. injection of heparin (250 iu). The trachea was then cannulated and the lungs were artificially ventilated (60 strokes min^{-1}) using a Ugo Basile Rodent ventilator. Systemic artery pressure was determined by inserting a cannula into the right carotid artery. The thorax was opened and pulmonary artery pressure was determined, in less than 1 min, by inserting a blunted hypodermic needle into the right ventricle and carefully advancing it into the main pulmonary artery as described in detail by Wanstall *et al*. (1995). Pressures were measured with a Bentley Trantec pressure transducer and were recorded on a Ugo Basile Gemini chart recorder. Mean pulmonary artery pressure and mean systemic artery pressure were taken as diastolic pressure + 1/3 (systolic pressure – diastolic pressure). Blood was then collected for measurement of haematocrit and the plasma angiotensinogen assays.

Rats were then used for one of the following procedures: (i) isolated pulmonary artery preparations (main and/or intralobar), (ii) isolated perfused lungs or (iii) histological analysis of lungs after fixation.

Hearts were removed from all rats except those used for the histological studies, and were divided into right ventricle (RV) and left ventricle plus septum (LV + S), blotted and weighed. Ratios of RV/body weight and (LV + S)/body weight were calculated. Body weights were: normoxic rats 301 ± 6.9 g ($n = 38$), hypoxic rats 247 ± 7.5 g ($n = 25$). Perindopril treatment had no effect on body weight in either group of rats. Note that in this study the ratio RV/(LV + S) was not considered an appropriate measure of right ventricular hypertrophy. This was because in some groups of rats perindopril treatment caused a significant reduction in (LV + S) (see Results).

Plasma angiotensinogen

Plasma angiotensinogen levels were measured by radioimmunoassay, as described in detail elsewhere (Thomas & Sernia, 1988). ^{125}I-labelled angiotensinogen and antibodies to angiotensinogen raised in sheep were used. Antibody-bound angiotensinogen was separated from free angiotensinogen by polyethylene glycol precipitation.

Isolated pulmonary artery rings

Main pulmonary artery Ring preparations (length 3 mm) of main pulmonary artery (i.d. 2–3 mm) were cleared of adventitia and mounted around two stainless steel wires in a vertical organ bath containing physiological salt solution (PSS) at 37°C and bubbled with 95% O_2 and 5% CO_2. The composition of the PSS was (mM): NaCl 118, KCl 5.9, $CaCl_2$ 1.5, $MgSO_4$ 0.72, $NaHCO_3$ 25, glucose 11.7, NaEDTA 0.025. The endothelium was left intact except in one series of experiments (see Experimental protocols). The resting forces for preparations from normoxic rats and hypoxic rats were 10 mN and 20 mN, respectively, approximately reflecting *in vivo* transmural pressures (Wanstall & O'Donnell, 1992; Wanstall *et al*., 1995). Active force was measured isometrically with either a Statham Universal transducer (UC3 + UL5) or a Grass FTO3 force displacement transducer attached to a micrometer (Mitutoyo, Tokyo, Japan).

At the conclusion of the experiment the following measurements were made; (a) the distance between the two stainless steel wires, and (b) the wet weight of the vessel, after blotting between filter paper for 30 s. The cross-sectional area in the plane perpendicular to the direction of applied force (defined as (2× vessel wall thickness × length of ring preparation)) was then calculated, as described by Wanstall & O'Donnell (1992), from the following formula:

$$\text{Cross-sectional area} = w(hd)^{-1}$$

Where h = the distance between the wires plus the diameter of the two wires (mm), w = wet weight (mg), and d = density = 1.06 mg mm^{-3} (Murphy, 1980). Since all preparations were the same length (3 mm), an increase in cross-sectional area reflected an increase in vessel wall thickness and hence hypertrophy of main pulmonary artery (Wanstall & O'Donnell, 1992).

Intralobar pulmonary artery One or two ring preparations (length 1.81 ± 0.03 mm, $n = 40$) of intralobar pulmonary artery (i.d. 440–750 μm), taken from the left lung lobe, were mounted on 40 μm diameter stainless steel wires in a small vessel myograph (Mulvany-Halpern type, Model 400A) containing PSS bubbled with 95% O_2 and 5% CO_2 at a temperature of 37°C. The resting forces (normoxic 1.91 ± 0.07 mN, $n = 23$; hypoxic 4.41 ± 0.19 mN, $n = 17$) were set to correspond approximately to *in vivo* pulmonary artery pressure and were individually determined for each preparation from passive length-tension curves (Myosight computer programme). No endothelium-denuded intralobar pulmonary artery preparations were studied because it is difficult to remove the endothelium from these vessels without damaging the underlying smooth muscle.

Isolated perfused lungs

The rat was exsanguinated *via* the vena cava. Cannulae were inserted into the pulmonary artery (*via* the right ventricle) and left atrium (*via* the left ventricle). The lungs were carefully removed and set up in a humidified chamber that was maintained at a temperature of 37°C. Lungs were ventilated *via* the previously inserted tracheal cannula (see above) with normoxic gas (20% O_2, 5% CO_2, balance N_2) using an Ugo Basile Rodent ventilator (60 strokes min^{-1}; inspiratory pressure 9 cmH_2O; end expiratory pressure 2.5 cmH_2O). Lungs were perfused *via* the pulmonary artery cannula initially at a constant flow rate (8 ml min^{-1}; Desaga peristaltic pump) with a modified PSS of the following composition (in mM): NaCl 119, KCl 4.7, $MgSO_4$ 1.17, $CaCl_2$ 3.2, KH_2PO_4 1.18, $NaHCO_3$ 22.6, glucose 5.5, sucrose 50. The perfusate also contained 4% w v^{-1} bovine serum albumin, 3 μM indomethacin and 2.5 nM angiotensin II. The effluent perfusate was collected *via* the atrial cannula and was not recirculated. Changes in perfusion pressure were measured *via* a side arm located in the perfusion line close to the pulmonary artery cannula and connected to a Bentley Trantec transducer.

Experimental protocols and expression of data

Pulmonary arteries After 1 h equilibration preparations were submaximally contracted with 0.1 μM phenylephrine (main pulmonary artery) or U46619 (intralobar pulmonary artery; 0.3 μM normoxic rats, 0.03 μM hypoxic rats). When the contraction had reached equilibrium a cumulative concentration-response (relaxation) curve, to acetycholine was obtained. In preparations in which the endothelium was removed, absence of a relaxant response to acetylcholine was taken as evidence for successful removal of the endothelium.

After washing the tissue, the PSS was replaced with K^+-depolarizing PSS (in which 80 mM NaCl was replaced with 80 mM KCl) in order to stabilize the preparations and provide a reference contraction. Preparations were washed again to return them to resting baseline force. Cumulative concentration-response (contraction) curves to one of the following contractile agents was then obtained: angiotensin I, angiotensin II, U46619 (thromboxane-mimetic) or 5-hydroxytryptamine (5-HT). In some experiments the tissue was washed, contracted again and a second contractile agent was tested. In those experiments where two contractile agents were tested on a single preparation, the particular agents examined and the order in which they were tested varied from preparation to preparation. When two intralobar pulmonary artery preparations were obtained from a single rat, different contractile agents were tested in the two preparations. In one series of experiments with angiotensin I and angiotensin II, perindoprilat (1 μM) was present 30 min before and also during the determination of the concentration-response curve; some of the main pulmonary artery preparations used in this series were endothelium-denuded. The concentration of perindoprilat used is of the same order of magnitude as the plasma concentrations achieved after oral administration of perindopril 10 or 30 mg kg^{-1} to rats (Jackson *et al*., 1988).

Contractile responses to 80 mM K^+ were measured as force (mN) and were expressed as tension (mN mm^{-1}, where mm = 2 × length of the preparation). Contractile responses to angiotensin I, angiotensin II, U46619 and 5-HT were expressed as a percentage of the reference contraction to K^+. Relaxant responses to acetylcholine were expressed as 'per cent reversal' of the induced submaximal contraction. All responses were plotted against drug concentration on a logarithmic scale. The potencies of the drugs were expressed as the negative log EC_{50}, where EC_{50} is the concentration producing 50% of the maximum response to the particular agent.

Isolated perfused lungs Lungs were allowed to equilibrate for 20 min at a flow rate of 8 ml min^{-1}. The flow rate was then increased to 20, 16 or 12 ml min^{-1}. The two lower flow rates were used in hypoxic rats (perindopril-treated and untreated respectively) and were selected so that perfusion pressures of 35 mmHg were not exceeded. This avoided the development of oedema. The flow rate was then reduced in a stepwise manner (2 ml min^{-1} decrements) down to a rate of 4 ml min^{-1} in order to obtain a passive pressure-flow relationship. The flow rate was then returned to 8 ml min^{-1} and two or three preliminary hypoxic pulmonary vasoconstrictor responses were obtained to establish reproducibility. These constrictor responses were induced by ventilating the lungs with a hypoxic gas mixture (2% O_2, 5% CO_2, balance N_2) rather than normoxic gas for 4 min. Four min of ventilation with normoxic gas separated successive hypoxic challenges. After this preliminary procedure a second pressure-flow relationship was obtained followed by a final hypoxic pulmonary vasoconstrictor response (at a flow rate of 8 ml min^{-1}) and these are the data presented in the Results section. Hypoxic pulmonary vasoconstrictor responses were measured in mmHg. Two measurements were recorded for each response, *viz*. the peak response (difference between peak pressure and resting pressure) and the equilibrium response (difference between pressure at equilibrium and resting pressure).

Mean pressure-flow plots were generated by linear regression using all the data points from the pressure-flow relationship from all of the lungs from each group of rats (Prism computer programme). From these plots two values were obtained, (i) the Y-intercept, which was determined by extrapolation and (ii) the slope. The Y-intercept represents the critical closing pressure, i.e. the minimum pressure that is required to keep the pulmonary vessels of the alveolar region open (Oddoy *et al*., 1991). The slope represents pulmonary vascular resistance (Oddoy *et al*., 1991).

Morphological measurements in histological sections of lungs

The pulmonary artery was cannulated, the left atrium was cut and the lung was perfused *via* this cannula with saline under a head of pressure (20, 30 or 40 cmH_2O) approximating the *in vivo* pulmonary artery pressure of the rats. The left atrium and pulmonary artery were then ligated to maintain the pressure within the pulmonary circulation. The lungs were subsequently fixed by perfusing them *via* the trachea with 10% formalin at a pressure of 25 cmH_2O for approximately 1 min. The trachea was tied off and the lungs and heart were removed *en bloc* and immersed in 10% formalin for 1 week. After fixation, longitudinal sections were taken from the left lobe and transverse sections were taken from each of the right lobes. All sections were 3 μm thick, were always taken from the same region of the left or right lobe and were stained with Miller's stain for elastin and Van Gieson's stain for smooth muscle.

Images of individual pulmonary arteries (mostly six, and never less than four, arteries per section) were captured using a video camera (Video 7, 8 bit 765 × 512 pixel CCD), mounted on a light microscope (Olympus BH5), linked to a computer (Macintosh Centris 650, with scion LG3 frame grabber). The longitudinal sections from the left lobe were examined at × 40 or × 100 magnification and provided images of vessels > 100 μm o.d. (range 101 – 500 μm). These vessels were in the

same region of the left lobe as the vessels taken for functional studies in the myograph. The transverse sections from the right lobes were examined at $\times 400$ magnification and provided images of vessels 30–100 μm o.d. Measurements of vessel diameter and medial wall thickness were obtained from these images using a computer programme (NIH image adapted for PC by Scion) and a method based on that of Morrell *et al.* (1995b). Vessel diameter was defined as the distance between two diametrically opposed external elastic laminae; two measurements were made, (i) along the line corresponding to the longest distance and (ii) along the line perpendicular to it, and these two values were averaged to give 'mean arterial diameter'. Medial wall thickness was defined and measured as the distance between internal and external elastic laminae; four measurements were made, one in each of the four quadrants defined by the perpendicular lines. Medial wall thickness was then expressed as a percentage of mean arterial diameter and termed per cent medial wall thickness'. Thus per cent medial wall thickness = (2 × medial wall thickness) × 100 ÷ vessel diameter.

Drugs

Angiotensin I (Auspep, Melbourne, Australia); angiotensin II (Sigma, St Louis, U.S.A.); acetylcholine chloride (Sigma); bovine serum albumin (Sigma); heparin (Fisons, Sydney, Australia); 5-hydroxytryptamine (5-HT; Sigma); indomethacin (Sigma); pentobarbitone sodium (Rhone Merieux, Brisbane, Australia); perindopril and perindoprilat (Gift from Servier Laboratories (Australia) Pty Ltd., Melbourne, Australia); phenylephrine (Sigma); U46619 (9, 11-dideoxy-11α, 9α-epoxymethano-prostaglandin $F_{2\alpha}$; Sigma).

Solutions of drugs were prepared as follows; angiotensin I (10 mM), angiotensin II (1 mM), phenylephrine (10 mM) in 0.01 M HCl; acetylcholine (10 mM), 5-HT (10 mM), perindoprilat (10 mM) in deionized water; indomethacin (10 mM), U46619 (10 mM) in absolute ethanol. Dilutions when required were made in PSS.

Statistical analyses

Mean values were calculated from data obtained in preparations from a number (n) of different animals and are quoted together with their s.e.mean. All values compared statistically were first shown to be normally distributed using the Kolmogorov-Smirnov test. Negative log EC_{50} values for angiotensins I and II in the presence and absence of perindoprilat were analysed by Student's t-test (unpaired values). Data obtained for mean systemic artery pressure and plasma angiotensingen levels in normoxic rats were analysed by a one-way analysis of variance (ANOVA) with a Dunnett's *post-hoc* test. All other values were analysed by two-way ANOVA with normoxic and hypoxic rats as one factor and perindopril treatment as the other factor. If there was a significant effect of hypoxic exposure a Student's t-test (unpaired values) was carried out in order to compare normoxic and hypoxic rats separately at each dose of perindopril. If there was a significant effect of perindopril treatment a one-way ANOVA with a Dunnet's *post-hoc* test was carried out separately for normoxic rats and hypoxic rats. If there was a significant interaction between the two factors, both of the above post tests were performed regardless of whether the two-way ANOVA indicated a significant effect of hypoxia or perindopril treatment. The statistics programmes that were used were Prism, Instat and Statistica.

Results

In vitro *effects of perindoprilat in pulmonary arteries from normoxic rats*

In both main and intralobar pulmonary arteries (endothelium-intact preparations) perindoprilat (1 μM; the active form of the prodrug perindopril) caused parallel shifts of the angiotensin I concentration-contraction curve to a higher concentration range (Figure 1). The shifts, measured at the level of the EC_{50}, were 2.62 and 2.00 log units in intralobar and main pulmonary arteries, respectively. In endothelium-denuded preparations of main pulmonary artery perindoprilat likewise shifted the angiotensin I curve (1.58 log units). Endothelium-denuded preparations of intralobar pulmonary arteries were not studied (see Methods). Perindoprilat had no effect on the concentration-response curves to angiotensin II (data not shown).

Choice of doses of perindopril for chronic administration to rats

Initial experiments were carried out in normoxic rats to determine appropriate doses of perindopril for the study. In these experiments the effects of 4 weeks of perindopril treatment on mean systemic artery pressure and plasma

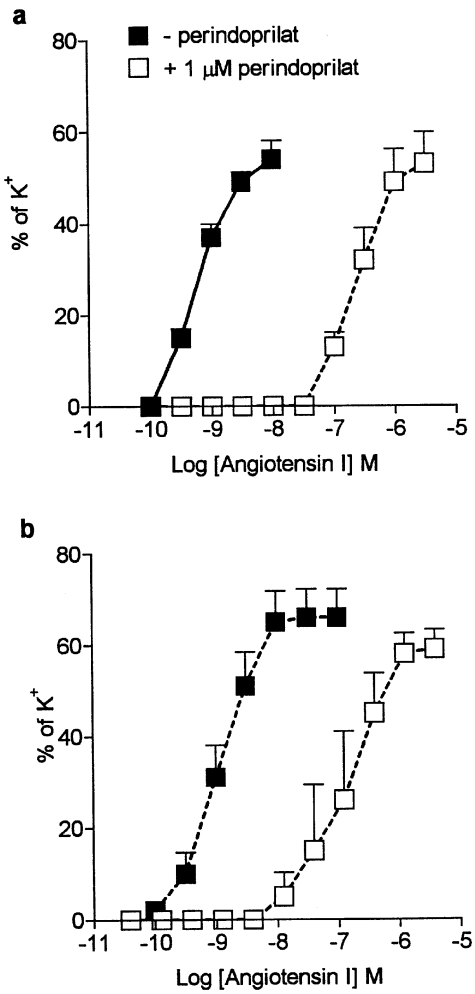

Figure 1 Concentration-response (contraction) curves to angiotensin I in the presence (open squares) and absence (closed squares) of 1 μM perindoprilat in intralobar (a) and main (b) pulmonary artery from normoxic rats ($n = 3-4$). Standard errors of mean responses are shown.

angiotensinogen levels were examined. Both of these parameters were significantly reduced in rats treated with doses of 3, 10 or 30 mg kg^{-1} d^{-1} perindopril, but not 0.3 mg kg^{-1} d^{-1} (Figure 2). A reduction in plasma angiotensinogen reflects inhibition of ACE because of a positive feedback link between angiotensin II and angiotensinogen (Johnston, 1990). The reduction in plasma angiotensinogen, but not the reduction in mean systemic artery pressure, was dose-dependent (Figure 2). From these data the doses selected for the remainder of the study were 3, 10 and 30 mg kg^{-1} d^{-1}.

Haemodynamic measurements, heart weights and haematocrit

Effects of exposure of rats to hypoxia Mean pulmonary artery pressure, RV/body weight and (LV + S)/body weight, but not mean systemic artery pressure, were significantly greater in hypoxic rats than in normoxic rats (Table 1). Haematocrit was also significantly increased (hypoxic rats $75 \pm 1.1\%$, $n = 25$; normoxic rats $43 \pm 0.8\%$, $n = 28$; $P < 0.001$). These data provided evidence for the presence of hypoxic pulmonary hypertension.

Effects of perindopril treatment In hypoxic rats treated with perindopril, there was a dose-related decline in mean pulmonary artery pressure (post test for linear trend, $P < 0.05$). At the highest dose (30 mg kg^{-1} d^{-1}) the reduction in pulmonary artery pressure was 18% ($P < 0.05$ if compared with hypoxic-untreated rats using Student's *t*-test) and at this dose there was also a significant reduction in RV/body weight (Table 1). Perindopril treatment had no effect on (LV + S)/body weight in hypoxic rats and only the highest dose caused any reduction in systemic artery pressure (24% reduction; Table 1). In normoxic rats the only effects of perindopril treatment were reductions in mean systemic artery pressure and (LV + S)/body weight. The reductions in systemic artery pressure, which were seen with all doses of perindopril, were 40–50%, i.e. much greater than the 24% reduction seen in hypoxic rats treated with 30 mg kg^{-1} d^{-1} (Table 1). Haematocrit values were not affected by perindopril treatment at any of the doses studied in either normoxic or hypoxic rats (data not shown).

Pulmonary vascular structure

Effects of exposure of rats to hypoxia In rats exposed to hypoxia there were changes in structure (vascular remodelling) throughout the pulmonary vascular tree, as follows. In perfused lungs there was an increase in the critical closing pressure (Y-intercept of passive pressure-flow plots) indicating vascular remodelling in the alveolar region (Table 2; Figure 3). It was noted that the slope of these plots was also increased representing an increase in pulmonary vascular resistance (Table 2; Figure 3). In pulmonary arteries 30–100 μm and

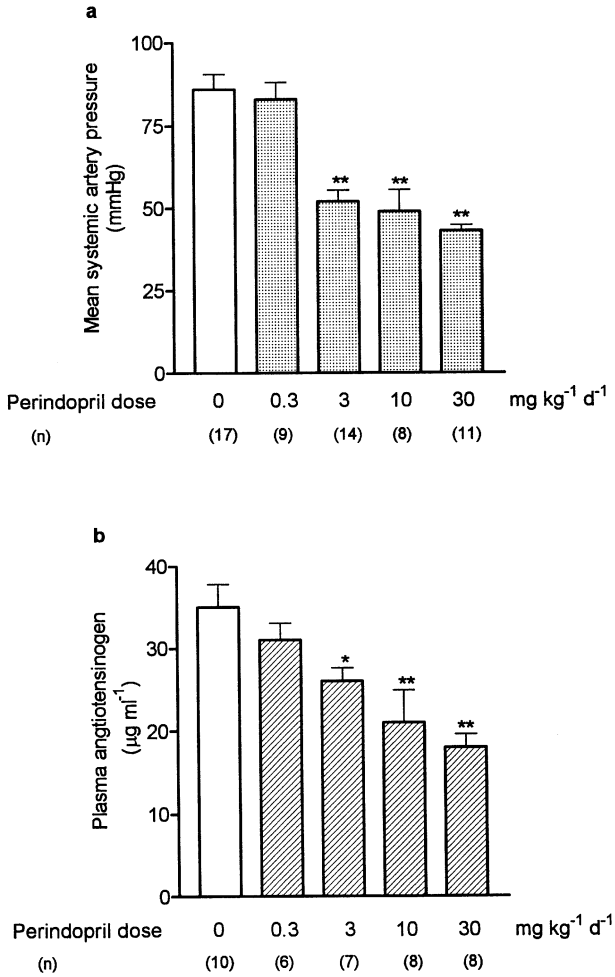

Figure 2 Mean systemic artery pressure (a) and plasma angiotensinogen levels (b) obtained in normoxic rats. Data for rats treated with perindopril at the doses indicated are shown by filled columns. Data for untreated rats (0 mg kg^{-1} d^{-1}) are shown by the open columns. Values are shown as means ± s.e.mean. n = number of rats. Asterisks – value significantly different from corresponding values for untreated rats:* $0.05 > P > 0.01$,** $0.01 > P > 0.001$.

Table 1 Mean pulmonary (MPAP) and systemic (MSAP) artery pressures and ratios of right ventricle (RV) and left ventricle plus septum (LV + S) to body weight in normoxic and hypoxic rats: effects of perindopril treatment

Perindopril dose (mg kg^{-1} d^{-1})	*MPAP* (mmHg)		*MSAP* (mmHg)		*RV/body weight* (mg g^{-1})		*(LV + S)/body weight* (mg g^{-1})	
	Normoxic	*Hypoxic*	*Normoxic*	*Hypoxic*	*Normoxic*	*Hypoxic*	*Normoxic*	*Hypoxic*
0	12 ± 1.0 (15)	28 ± 1.6*** (17)	86 ± 4.6 (17)	87 ± 6.2 (13)	0.64 ± 0.02 (30)	1.8 ± 0.06*** (19)	2.5 ± 0.04 (30)	3.0 ± 0.10*** (19)
3	12 ± 0.7 (14)	26 ± 1.5*** (15)	52 ± 3.5## (14)	79 ± 3.8*** (14)	0.59 ± 0.02 (10)	1.9 ± 0.08*** (8)	2.1 ± 0.05## (10)	2.9 ± 0.13*** (8)
10	12 ± 0.9 (8)	25 ± 1.3*** (7)	49 ± 6.6## (8)	90 ± 4.6*** (6)	0.63 ± 0.01 (4)	1.7 ± 0.10*** (4)	1.9 ± 0.02## (4)	2.6 ± 0.13*** (4)
30	12 ± 0.7 (12)	23 ± 1.2*** (16)	43 ± 1.8## (11)	66 ± 5.7**# (14)	0.57 ± 0.03 (8)	1.5 ± 0.08***# (12)	2.0 ± 0.06## (8)	2.8 ± 0.14*** (12)

Values are mean ± s.e.mean. Number of rats in parentheses. Asterisks – values significantly different from corresponding values for normoxic rats: **$0.01 > P > 0.001$, ***$P < 0.001$. Hashes – values significantly different from corresponding values for rats not treated with perindopril (0 mg kg^{-1} d^{-1}): #$0.05 > P > 0.01$, ## $0.01 > P > 0.001$.

Table 2 Y-intercept and slope of pressure-flow plots in lungs from normoxic and hypoxic rats: effects of perindopril treatment of rats

Perindopril Dose (mg kg^{-1} d^{-1})	*Y-intercept* (mmHg)[a]		*Slope (mmHg ml^{-1} min^{-1})*[b]	
	Normoxic	*Hypoxic*	*Normoxic*	*Hypoxic*
0	5.7±0.49 (7)	13.8±2.50** (6)	0.65±0.04 (7)	1.30±0.29* (6)
3	5.1±0.94 (4)	13.4±2.68* (7)	0.60±0.07 (4)	1.56±0.28* (7)
10	7.5±1.21 (4)	9.7±1.60 (4)	0.57±0.09 (4)	1.08±0.15* (4)
30	7.3±0.39 (3)	9.7±1.26 (4)	0.52±0.03 (3)	1.08±0.12* (4)

Values are mean±s.e.mean. Number of lungs in parentheses: [a]Y-intercept represents critical closing pressure. [b]Slope represents pulmonary vascluar resistance. Asterisks – values significantly different from corresponding values for normoxic rats *$0.05 > P > 0.01$, **$0.01 > P > 0.001$.

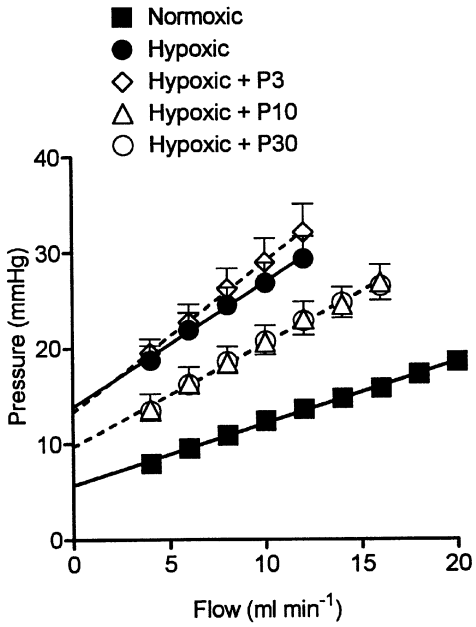

Figure 3 Pressure-flow plots obtained in isolated perfused lungs from untreated normoxic and hypoxic rats, and hypoxic rats treated with perindopril (P; 3, 10 and 30 mg kg^{-1} d^{-1}). Values are means±s.e.mean. For *n* values see Table 2.

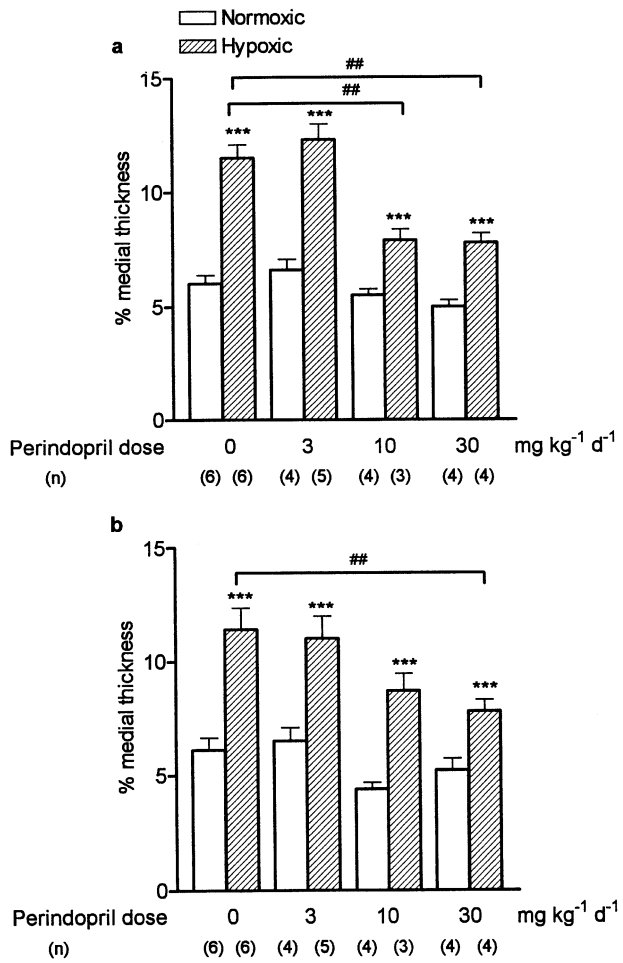

Figure 4 Per cent medial wall thickness, of pulmonary arteries 30–100 μm o.d. (a) and 101–500 μm o.d. (b) from normoxic (open columns) and hypoxic rats (hatched columns), either untreated (0 mg kg^{-1} d^{-1}) or perindopril-treated (3, 10 or 30 mg kg^{-1} d^{-1}). Values are means±s.e.mean. *n*=number of rats. *** Significantly different from corresponding values for normoxic rats $P<0.001$. ## Significantly different from corresponding values for untreated hypoxic rats $0.01 > P > 0.001$.

101–500 μm o.d. (examined in histological sections of lungs) values of 'per cent medial wall thickness' were approximately double those seen in normoxic rats (Figure 4). In main pulmonary artery (2–3 mm i.d.) there was a significant increase in calculated 'cross-sectional area', a measurement that reflects vessel wall thickness (hypoxic rats, 0.93±0.05 mm^2, $n=13$; normoxic rats, 0.71±0.04 mm^2, $n=26$; $0.01 > P > 0.001$).

Effects of perindopril treatment Treatment of hypoxic rats with perindopril (10 or 30 but not 3 mg kg^{-1} d^{-1}) attenuated the above changes in pulmonary vascular structure in all vessels studied except main pulmonary artery. Pressure-flow plots in perfused lungs were shifted towards that seen in lungs from normoxic rats (Figure 3). Consequently at doses of 10 and 30 mg kg^{-1} d^{-1} there was no longer a significant difference in critical closing pressure between normoxic and hypoxic rats (Table 2). In addition these doses caused a small, but non-significant, reduction in the slope of the pressure-flow plots (Table 2). Values of per cent medial wall thickness of pulmonary arteries 30–100 μm and 101–500 μm were also reduced in perindopril-treated hypoxic rats (Figure 4). In vessels 30–100 μm doses of 10 and 30 mg kg^{-1} d^{-1} were effective whereas in vessels >100 μm only the highest dose of perindopril (30 mg kg^{-1} d^{-1}) had a significant effect. Despite these significant effects of perindopril treatment, the values of per cent medial wall thickness were still greater in hypoxic rats than normoxic rats although this difference was less pronounced than in untreated rats (Figure 4). Cross sectional

area of main pulmonary artery was not affected by perindopril treatment of hypoxic rats, even at the highest dose (hypoxic perindopril-treated rats, 0.90 ± 0.06 mm^2, $n=7$; normoxic perindopril-treated rats, 0.64 ± 0.06 mm^2, $n=8$; $P>0.05$ compared with values in untreated rats cited above). In normoxic rats, in contrast to hypoxic rats, perindopril treatment had no significant effect on any of the measures of pulmonary vascular structure (Table 2; Figures 3 and 4).

Pulmonary vascular function

Effects of exposure of rats to hypoxia The functional responses studied were: (i) in isolated perfused lungs, hypoxic pulmonary vasoconstriction and (ii) in intralobar and main pulmonary arteries, contractions to several vasoconstrictor spasmogens and relaxation to an endothelium-dependent vasodilator. In preparations from hypoxic rats the following alterations in pulmonary vascular function were observed.

Hypoxic pulmonary vasoconstrictor responses in lungs fom hypoxic rats were significantly increased when compared with data in normoxic rats. The character of these responses was also different, i.e. in hypoxic rats responses generally reached a peak within 2 min and subsequently declined to equilibrium whereas in normoxic rats the difference between peak and equilibrium values was minimal (Figure 5, Table 3). The size of the reference contraction induced by depolarization with 80 mM K^+ (expressed as mN mm^{-1}) was significantly increased in both intralobar (hypoxic 2.0 ± 0.17, $n=12$; normoxic, 1.5 ± 0.07, $n=18$; $0.01>P>0.001$) and main pulmonary arteries (hypoxic 6.3 ± 0.31, $n=13$; normoxic 3.9 ± 0.16, $n=26$; $P<0.001$). Contractile responses to 5-HT in isolated arteries were also significantly enhanced. This was seen as an increase in potency in vessels of both sizes (Table 4) and also as an increase in maximum contraction, relative to K^+, in intralobar pulmonary artery (Figure 6). The maximum contractile response (relative to K^+) to U46619, studied in intralobar pulmonary arteries only, was also markedly increased but potency was unchanged (Table 4; Figure 7). In contrast to the above increases in response, contractile responses to angiotensins I and II were reduced. This was seen as reductions in potency in vessels of both sizes and reductions in maximum response in main pulmonary artery (Table 4; Figures 8 and 9). The only response that was not different in hypoxic and normoxic rats was the relaxant response to acetycholine. Values of potency (negative log EC_{50}) and maximum response (as per cent reversal of induced contraction) for acetylcholine were: intralobar pulmonary artery, hypoxic 6.59 ± 0.08, $68 \pm 3.2\%$, $n=8$; normoxic 6.75 ± 0.14, $73 \pm 3.2\%$, $n=5$; main pulmonary artery, hypoxic 7.16 ± 0.16, $68 \pm 5.2\%$, $n=10$; normoxic 7.41 ± 0.14, $60 \pm 7.2\%$, $n=5$.

Effects of perindopril treatment Based on the effects of perindopril on pulmonary vascular remodelling in hypoxic rats, a dose of 30 mg kg^{-1} d^{-1} was chosen for the experiments in which functional responses were studied. Perindopril treatment of normoxic rats had no effect on hypoxic pulmonary vasoconstriction. However the exaggerated hypoxic pulmonary vasoconstrictor response seen in lungs from hypoxic rats was attenuated by perindopril treatment and, as a result, the response (at equilibrium) was restored to the value seen in normoxic rats (Table 3). In contrast, the changes in functional responses to K^+-depolarization, 5-HT, U46619 and angiotensins I and II seen in intralobar and main pulmonary arteries from hypoxic rats (see above) were not prevented or attenuated by perindopril treatment; hence differences between normoxic and hypoxic rats were still apparent (Table 4;

Table 3 Hypoxic pulmonary vasoconstrictor responses (peak and equilibrium) obtained in lungs from normoxic and hypoxic rats: effect of perindopril treatment of rats

Perindopril dose (mg kg^{-1} d^{-1})	*Peak response* (mmHg)		*Equilibrium response* (mmHg)	
	Normoxic	*Hypoxic*	*Normoxic*	*Hypoxic*
0	4.1 ± 0.9 (6)	20.6 ± 5.2** (6)	3.5 ± 0.5 (6)	13.7 ± 2.4** (6)
30	5.0 ± 1.3 (3)	12.6 ± 3.8 (4)	4.2 ± 1.2 (3)	4.9 ± 0.4# (4)

Values are mean $\pm$ s.e.mean. Number of lungs in parentheses. **Significantly different from corresponding values for normoxic rats $0.01>P>0.001$. #Significantly different from corresponding value for untreated hypoxic rats $0.05>P>0.01$.

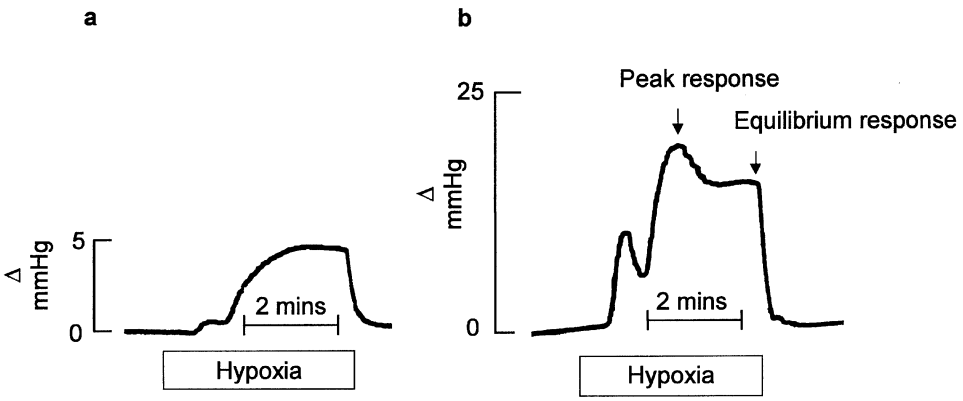

Figure 5 Original tracings showing hypoxic pulmonary vasoconstrictor responses obtained in isolated perfused lungs from untreated normoxic (a) and untreated hypoxic (b) rats. Hypoxia = ventilation with 2% O_2. Once the response had reached equilibrium, ventilation with hypoxic gas was replaced with normoxic gas; pressure then returned towards resting levels. In lungs from hypoxic but not normoxic rats the response to hypoxia reached a peak and subsequently declined to equilibrium. Note the different scales on the pressure axes in (a) and (b).

Table 4 Potency of 5-HT, U46619, angiotensin I (AI) and angiotensin II (AII) in isolated pulmonary arteries from normoxic and hypoxic rats: effects of perindopril treatment of rats

Drug	*Perindopril dose* (mg kg^{-1} d^{-1})	*Potency (negative log EC_{50})* *Intralobar pulmonary artery* *Normoxic*	*Hypoxic*	*Main pulmonary artery* *Normoxic*	*Hypoxic*
5-HT	0	5.21±0.16 (5)	6.22±0.07*** (8)	5.10±0.08 (7)	5.84±0.07*** (5)
	30	4.85±0.07 (4)	5.92±0.09***# (4)	4.74±0.16 (4)	5.84±0.11** (3)
U46619	0	7.47±0.10 (6)	7.62±0.10 (4)	ND	ND
	30	7.42±0.11 (4)	7.52±0.04 (3)	ND	ND
AI	0	9.41±0.13 (5)	8.87±0.12* (4)	8.81±0.10 (9)	8.14±0.16** (5)
	30	8.81±0.07## (4)	8.37±0.14*# (5)	7.73±0.02## (4)	7.46±0.06**## (4)
AII	0	9.78±0.11 (8)	9.26±0.16* (8)	8.86±0.11 (10)	8.14±0.13** (5)
	30	10.11±0.17 (6)	9.31±0.23* (4)	8.66±0.04 (4)	8.13±0.09** (4)

Values are mean±s.e.mean. Number of rats in parentheses. ND = not determined. Asterisks – values significantly different from corresponding values for normoxic rats: *$0.05 > P > 0.01$, **$0.01 > P > 0.001$, ***$P < 0.001$. Hashes – values significantly different from corresponding values for rats not treated with perindopril (0 mg kg^{-1} d^{-1}): #$0.05 > P > 0.01$, ##$0.01 > P > 0.001$.

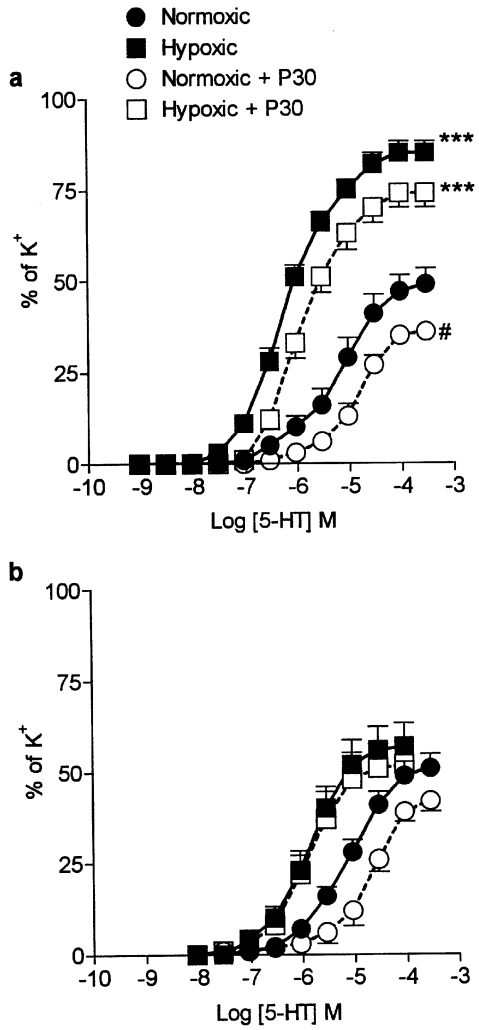

Figure 6 Concentration-response (contraction) curves to 5-HT in intralobar (a) and main (b) pulmonary arteries from normoxic and hypoxic rats, either untreated or treated with perindopril (P; 30 mg kg^{-1} d^{-1}). Standard errors of mean responses are shown. For *n* values see Table 4. *** Significant difference between value in hypoxic rats and corresponding value in normoxic rats $P < 0.001$ #Significant difference between value in perindopril-treated rats and corresponding value in untreated normoxic rats $0.05 > P > 0.01$.

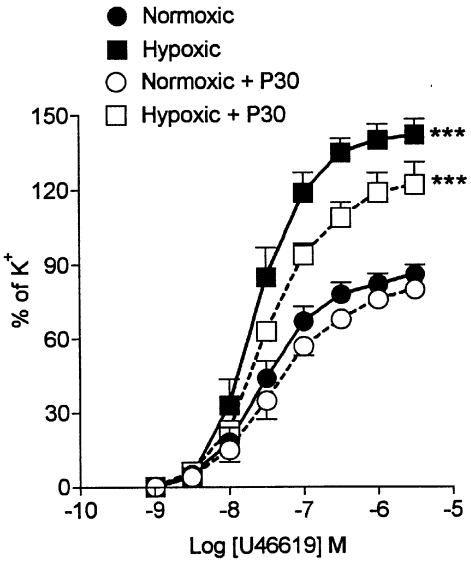

Figure 7 Concentration-response (contraction) curves to U46619 in intralobar pulmonary artery from normoxic and hypoxic rats, either untreated or perindopril treated (*P*; 30 mg kg^{-1} d^{-1}). Standard errors of mean responses are shown. For *n* values see Table 4. ***Significant difference between value in hypoxic rats and corresponding value in normoxic rats $P < 0.001$.

Figures 6–9). Perindopril treatment of both normoxic and hypoxic rats had no effect on potency or maximum response to acetycholine (data not shown).

There were two effects of perindopril treatment on pulmonary vascular function that require comment because these effects were seen in both normoxic and hypoxic rats. Firstly, the potency of angiotensin I was reduced reflecting the inhibition of tissue ACE in these arteries (Table 4). Secondly there was a small depressant effect on responses to 5-HT in intralobar pulmonary artery, seen as either a decrease in maximum (normoxic rats; Figure 6) or a decrease in potency (hypoxic rats; Table 4). However it should be emphasized that perindopril treatment did not prevent the enhancement of 5-HT responses or the depression of angiotensin I responses induced by exposure of rats to hypoxia (Table 4; Figures 6 and 8).

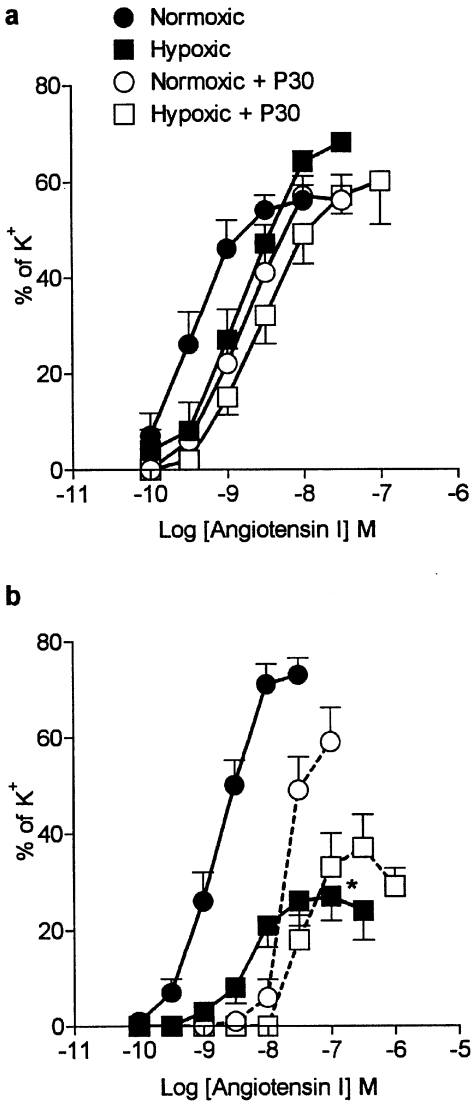

Figure 8 Concentration-response (contraction) curves to angiotensin I in intralobar (a) and main (b) pulmonary arteries from normoxic and hypoxic rats, either untreated or perindopril treated (P; 30 mg kg^{-1} d^{-1}). Standard errors of mean responses are shown. For *n* values see Table 4. *Significant difference between value in hypoxic rats and corresponding value in normoxic rats $0.05 > P > 0.01$.

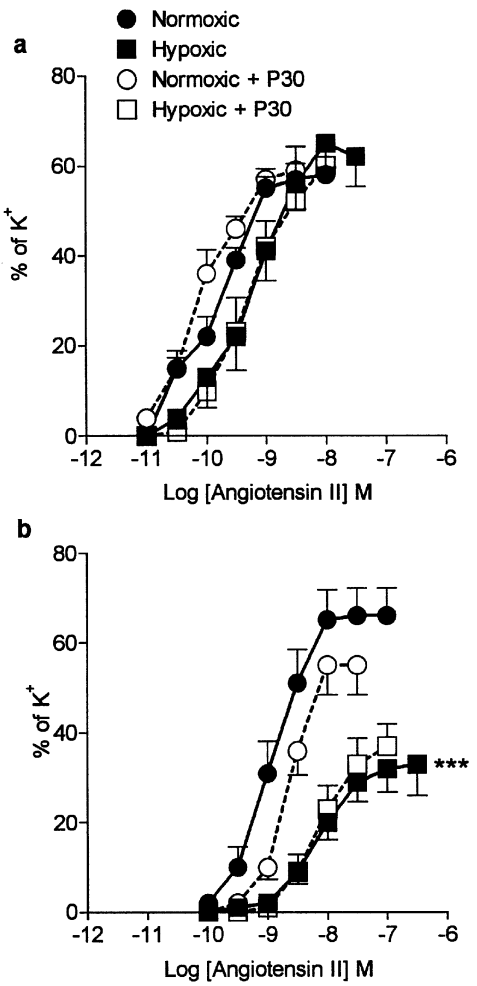

Figure 9 Concentration-response (contraction) curves to angiotensin II in intralobar (a) and main (b) pulmonary arteries from normoxic and hypoxic rats, either untreated or perindopril treated (P; 30 mg kg^{-1} d^{-1}). Standard errors of mean responses are shown. For *n* values see Table 4. ***Significant difference between value in hypoxic rats and corresponding value in normoxic rats $P < 0.001$.

Discussion

In this study we have characterized, for the first time, the effect of the ACE inhibitor, perindopril, on various features of hypoxic pulmonary hypertension in rats. There are several novel aspects of this study. First, and most importantly, the study has evaluated the effects of perindopril treatment not only on the changes in pulmonary vascular structure (vascular remodelling) seen in pulmonary hypertension, but also on alterations in pulmonary vascular function. This has not been investigated previously for any ACE inhibitor or for other types of anti-remodelling drug (e.g. endothelin antagonists). Second, the effect of perindopril on pulmonary vascular remodelling has been assessed at all levels of the vascular tree, from main pulmonary artery down to the alveolar vessels. Third, in the studies on pulmonary vascular structure, a range of doses of perindopril has been investigated allowing us to determine the minimum and maximum effective doses on this feature of pulmonary hypertension. Previous studies on the effects of ACE inhibitors on vascular remodelling in rats with pulmonary hypertension have mostly examined a limited range of vessel sizes and have generally used only a single dose of drug.

There were no previous studies of perindoprilat on isolated pulmonary arteries. Hence, in initial experiments the *in vitro* effects of 1 μM perindoprilat on responses to angiotensin I and angiotensin II in isolated pulmonary arteries were examined. In both intralobar and main pulmonary artery perindoprilat selectively reduced the potency of angiotensin I without affecting responses to angiotensin II. This indicated that conversion of angiotensin I to angiotensin II in rat pulmonary arteries is effectively inhibited by this particular ACE inhibitor. Removal of the endothelium from main pulmonary artery did not alter responses to angiotensin I or prevent the shift in the angiotensin I curve by perindoprilat. From this we conclude that conversion of angiotensin I to angiotensin II in rat pulmonary artery occurs at sites other than the endothelium, confirming the observations of Ito *et al.* (1988). Since the adventitia was largely removed from the preparations, the most likely site is the smooth muscle.

The selection of a suitable dose of perindopril for chronic administration to rats was based on preliminary experiments in

which the effects on systemic artery pressure and plasma angiotensinogen levels in normoxic rats were studied. Both of these parameters are typically reduced by ACE inhibitors (Vanhoutte *et al*., 1989; Johnston, 1990) and in the present study were reduced by doses of perindopril $\geqslant 3$ mg kg^{-1} d^{-1}. Therefore this was the lowest dose used in the pulmonary hypertension studies. The reduction in systemic artery pressure by perindopril at a dose of 3 but not 0.3 mg kg^{-1} d^{-1} agrees with the findings of Minami & Head (1993). Retrospective analysis of the data in isolated artery preparations showed that the potency of angiotensin I, but not angiotensin II, was reduced in intralobar and main pulmonary arteries from rats treated for 4 weeks with perindopril (30 mg kg^{-1} d^{-1}). This provided indirect evidence that perindopril administered chronically to rats *in vivo* once daily was able to inhibit ACE activity in the pulmonary vasculature.

In rats exposed to hypoxia for 4 weeks mean pulmonary artery pressure was more than double that seen in normoxic rats and there was marked right ventricular hypertrophy. In these hypoxic rats perindopril at the highest dose studied (30 mg kg^{-1} d^{-1}) caused a modest reduction in pulmonary artery pressure. This is comparable to findings for other ACE inhibitors in chronically hypoxic rats, e.g. captopril (Morrell *et al*., 1995b) and quinapril (Nong *et al*., 1996), and better than results with cilazapril which caused no reduction in pulmonary artery pressure at all (Clozel *et al*., 1991). The small reduction in pulmonary artery pressure in the present study cannot be attributed to a direct vasodilator action on the pulmonary circulation since in normoxic rats pulmonary artery pressure was not reduced by perindopril. In hypoxic rats perindopril also reduced right ventricular hypertrophy which is most likely due to the reduction in after-load to the heart as a result of reduced pulmonary artery pressure. However a direct effect of perindopril on inhibiting ACE in the right ventricle cannot be ruled out since ACE expression has been shown to be increased in the right ventricle of chronically hypoxic rats (8 and 14 days exposure; Morrell *et al*., 1997).

The rise in pulmonary artery pressure associated with pulmonary hypertension is considered to be due in part to pulmonary vascular remodelling. In this study exposure of rats to hypoxia caused pulmonary vascular remodelling at all levels of the vascular tree and this is consistent with the findings of others (e.g. Emery *et al*., 1981; Morrell *et al*., 1995b; Jeffery & Wanstall, 1998b). There was an increase in the critical closing pressure of isolated perfused lungs and this indicated the extension of smooth muscle into the very small (<50 μm o.d.), normally non-muscular arteries of the alveolar region (Oddoy *et al*., 1991). There was also an increase in the per cent medial wall thickness of intralobar pulmonary arteries (30–100 and 101–500 μm o.d.) which indicates medial hypertrophy/hyperplasia in these more proximal muscular arteries. Likewise, in main pulmonary arteries (2–3 mm i.d.) there was medial hypertrophy as seen by an increase in 'cross-sectional area' (which represents an increase in vessel wall thickness).

When rats exposed to hypoxia were given perindopril at a dose of 3 mg kg^{-1} d^{-1} there was no effect on any of these measurements of pulmonary vascular remodelling. This is in contrast to findings in systemic vessels. For example, perindopril at doses less than 2 mg kg^{-1} d^{-1} reduced (i) the medial thickness of descending thoracic aorta from rats with experimental systemic hypertension (Levy *et al*., 1989) and (ii) hypertrophy of mesenteric artery and aorta in a rat model of diabetes (Vranes *et al*., 1995). Hence it appears that remodelling of pulmonary vessels may be more resistant to ACE inhibitor therapy than remodelling in the systemic circulation.

A dose of 10 mg kg^{-1} d^{-1} perindopril was successful in reducing vascular remodelling but only in the alveolar vessels (perfused lung experiments) and in the smaller of the intralobar vessels assessed histologically (i.e. those <100 μm o.d.). In these vessels 30 mg kg^{-1} d^{-1} was no more effective than 10 mg kg^{-1} d^{-1}; furthermore, although the remodelling was markedly inhibited it was not totally prevented. In the larger intralobar vessels (101–500 μm o.d), only the highest dose (30 mg kg^{-1} d^{-1}) significantly reduced remodelling, and in main pulmonary artery none of the doses of perindopril had any measurable effect. Together these data suggest that (i) the minimum effective dose of perindopril to inhibit vascular remodelling in hypoxic pulmonary hypertension in rats is between 3 and 10 mg kg^{-1} d^{-1}, (ii) the effect of 30 mg kg^{-1} d^{-1} may be maximal, (iii) other mitogenic/hypertrophic factors (e.g. endothelin, Cacoub *et al*., 1993, and growth factors, Bishop *et al*., 1995), in addition to angiotensin II, probably contribute to the remodelling and (iv) the effectiveness of perindopril is dependent on vessel size, the smaller the vessel the greater the effect.

The contrasting effects of perindopril on pulmonary vessels of different sizes reflects findings with one other ACE inhibitor (captopril; Morrell *et al*., 1995b), but the reason for this variability is presently unknown. It may be related to the finding that in hypoxic pulmonary hypertension, ACE expression is increased in the walls of small, but not large, pulmonary arteries (Morrell *et al*., 1995a). Another possibility is that angiotensin II may be particularly important for remodelling in the smaller vessels whereas other mitogens may play a greater role in the larger vessels. The lack of effect of perindopril treatment on hypertrophy of main pulmonary artery is not due to failure of the perindopril treatment to inhibit ACE in this conduit artery. This is because in main pulmonary artery from perindopril-treated rats there was a reduction in the potency of angiotensin I comparable to that seen in intralobar arteries (approximately 10 fold reduction). Remodelling in the larger vessels may reflect hypertrophy of existing smooth muscle cells rather than development of new smooth muscle cells, as occurs in the smaller vessels (Gillespie *et al*., 1995); whether this can explain the lack of effect of perindopril treatment on remodelling of main pulmonary artery is presently unknown.

Having established that perindopril could attenuate the development of pulmonary vascular remodelling, the question of whether it had any effect on altered pulmonary vascular function in hypoxic rats was addressed. A variety of alterations in pulmonary vascular function were observed in intralobar and main pulmonary arteries from hypoxic rats. These included increases in the contractile responses (maximum and/or potency) to K^+, 5-HT and U46619 (thromboxane mimetic). An increase in the size of the hypoxic pulmonary vasoconstrictor response in isolated perfused lungs from hypoxic rats was also observed. Some of these increases in response have been described in previous studies on hypoxic rats (Bee & Wach, 1984; Homer *et al*., 1996; MacLean *et al*., 1996; Jeffery & Wanstall, 1998b). In contrast, there were reductions in responses to angiotensin II (and corresponding reductions in responses to angiotensin I) in both main and intralobar pulmonary arteries from hypoxic rats. This finding is comparable to results in vessels from rats with monocrotaline-induced pulmonary hypertension (Altiere *et al*., 1986; Madden *et al*., 1994) but, as far as we know, reduced responsiveness to these peptides has not previously been described in isolated pulmonary arteries from hypoxic rats. On the contrary, in perfused lungs from rats exposed to hypoxia for 2 weeks, vasoconstrictor responses to angiotensins I and II

were increased (Emery *et al*., 1981; Russell *et al*., 1990). The reason for the reduction in contractile responses to angiotensin II is not known. One possibility that was considered was an upregulation of vasodilator AT_2 receptors. However this possibility was dismissed because the selective AT_2 receptor antagonist, PD123319, did not enhance the angiotensin II contractions in main or intralobar pulmonary arteries from either hypoxic or normoxic rats (Widdop & Wanstall, unpublished data).

When rats were treated with perindopril (30 mg kg^{-1} d^{-1}), none of the functional changes seen in isolated arteries from hypoxic rats was prevented. This was despite the fact that this dose of perindopril attenuated remodelling in vessels of the same size as those in which functional responses were measured (i.e. intralobar pulmonary arteries >100 μm). Therefore we conclude that vascular remodelling and altered vascular function are not necessarily related and that even if vascular remodelling can be attenuated or prevented by appropriate drug treatment, abnormal pulmonary vasoconstriction may still persist. Interestingly, treatment of rabbits with carotid atheroma lesions with 0.3 mg kg^{-1} d^{-1} perindopril did not prevent the increase in sensitivity to 5-HT in carotid arteries even though the atheromatous lesions were reduced in size (Dusting *et al*., 1995).

The increase in the hypoxic pulmonary vasoconstrictor response in perfused lungs from hypoxic rats was the only alteration in pulmonary vascular function that was prevented by perindopril treatment. ACE inhibitors do not directly inhibit this response since perindopril (present study) and other ACE inhibitors (Prewitt & Leffler, 1981; Morrell *et al*., 1995b) do not reduce hypoxic pulmonary vasoconstriction in normoxic rats. Thus our data indicate that augmented hypoxic pulmonary vasoconstriction in pulmonary hypertension is a direct reflection of pulmonary vascular remodelling, particularly the extension of smooth muscle into small alveolar arteries. The only other effects of perindopril treatment on pulmonary vascular function were not confined to hypoxic rats and therefore cannot be viewed as preventing a change associated with pulmonary hypertension. These effects, seen in control as well as hypoxic rats, were (i) a small depression in responses to 5-HT, for which we do not presently have any explanation and (ii) the anticipated reduction in responses to angiotensin I.

Taken together the findings on pulmonary vascular structure and function may explain, at least in part, why perindopril had only a modest effect on pulmonary artery pressure in hypoxic rats. The fact that pulmonary artery pressure remained somewhat elevated probably reflects the continuing abnormal vasoconstriction and the small amount of residual remodelling. Increased blood viscosity may have been another contributing factor because the haematocrit remained elevated in perindopril-treated hypoxic rats. The possibility that administration of perindopril in divided doses, i.e. two or three times a day instead of once daily, would have had more profound effects on any of the parameters studied cannot be excluded.

The measurements of systemic artery pressure provided data that warrants comment. In hypoxic rats systemic artery pressure was reduced by only the highest dose of perindopril whereas in normoxic rats all doses had an effect. Furthermore this reduction was much less than that seen in normoxic rats. A similar result has been reported with other ACE inhibitors in hypoxic rats (Clozel *et al*., 1991; Morrell *et al*., 1995b; Nong *et al*., 1996) although in most of these studies corresponding data in normoxic rats is not provided. The reason why perindopril had less effect on systemic artery pressure in hypoxic than in normoxic rats is not known but it may be due to down-regulation of the circulating, as opposed to the tissue, renin-angiotensin system by hypoxia. This is supported by the finding that plasma angiotensinogen levels are reduced by as much as 75% in rats exposed to hypoxia (Jeffery & Wanstall, unpublished; Martin *et al*., 1987). If the same occurs in humans with hypoxic pulmonary hypertension, systemic hypotension may not constitute a problem if these patients are treated with ACE inhibitors.

In conclusion our study supports the concept that angiotensin II is involved in the pulmonary vascular remodelling that is associated with pulmonary hypertension. The data confirm that inhibition of ACE can attenuate this particular pathological change, and our study shows that perindopril treatment is most effective in the smaller vessels. However, treatment with an ACE inhibitor does not prevent the changes in pulmonary vascular function that occur in pulmonary hypertension with the exception of the increase in hypoxic pulmonary vasoconstriction. The data from this study have therefore provided evidence that altered vascular function is largely independent of vascular remodelling. Hence, as a general principle, abnormal pulmonary vasoconstriction may still persist even if vascular remodelling can be prevented by drug treatment. Whether a better effect on pulmonary artery pressure in pulmonary hypertension can be achieved by combining an anti-remodelling drug (e.g. an ACE inhibitor) with a drug that can oppose abnormal vasoconstriction (e.g. a vasodilator drug) is currently under investigation.

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