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# The regulation of pulmonary vascular tone

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**1** The ability to manipulate pharmacologically pulmonary vascular tone independent of effects on systemic blood vessels is a desirable objective. Elucidation of the biochemical mechanisms underlying hypoxia-induced pulmonary vasoconstriction (HPV) may permit preferential targeting of the pulmonary circulation.

**2** Here we review our studies of the role of locally synthesized candidate vasoactive factors in HPV. In addition, we present data demonstrating an attenuated pressor response to hypoxia in the pulmonary circulation of Fischer 344 rats compared with the Wistar-Kyoto (WKY) rat strain.

**3** We propose that a systematic genome-wide search using the HPV phenotype and a panel of highly informative microsatellite markers will elucidate the genetic loci underlying the difference in susceptibility to HPV in these two rat strains and provide a valuable and novel insight into the factors that determine the HPV response.

**Keywords** hypoxia vasoactive factors genetics rat strains

## The clinical problem

The normal adult pulmonary circulation is a low-pressure, low-resistance system with little or no resting vascular tone. Pulmonary artery pressure may be increased, however, in a number of clinical situations, such as in patients with chronic hypoxic lung disease or a left-to-right intracardiac shunt, and, less frequently, as a primary event. In the long-term, pulmonary hypertension leads to right ventricular hypertrophy, heart failure and premature death.

The current treatment of pulmonary hypertension is unsatisfactory [1, 2]. Oxygen is an effective pulmonary vasodilator in pulmonary hypertension secondary to chronic obstructive lung disease [3] and has been reported to be beneficial in other forms of pulmonary hypertension [4, 5], but it is inconvenient to administer, relatively expensive and has to be given for at least 12 h per day. A number of more conventional vasodilators have been examined, but the doses required to reduce the elevated pulmonary artery pressure are usually so high that they are associated with symptomatic and unacceptable falls in systemic blood pressure. Prostacyclin [6] and adenosine [7] infused directly into the pulmonary artery and inhaled nitric oxide [8] show some selectivity for the pulmonary circulation by virtue of the short half-life of the active compound in the systemic circulation, but none of these therapies is optimal. There are obvious practical difficulties with the chronic infusion of compounds and there are concerns about the toxicity of chronic nitric oxide administration. Clearly, the treatment of pulmonary hypertension would benefit from a more comprehensive range of drugs that target selectively the pulmonary circulation.

## Hypoxia-induced pulmonary vasoconstriction (HPV)

Oxygen tension is a major regulator of pulmonary vascular tone. Ventilation of lungs with an hypoxic gaseous mixture (i.e. decreasing $PO_2$ from $\sim$130 to 30–40 mmHg) leads to acute pulmonary vasoconstriction, most pronounced in precapillary arterioles [9]. Constriction is also observed in isolated pulmonary arteries (the degree of vasoconstriction correlating inversely with diminishing vessel size) [10–12] and isolated pulmonary vascular smooth muscle cells [11] perfused at low oxygen tension ($\sim$40 mmHg).

In contrast, systemic vessels in the intact rat [13], mesenteric resistance vessels perfused *in situ* [14] and in an organ bath [10, 12] and systemic vascular smooth muscle cells in culture [11] relax when exposed to comparable hypoxic conditions. Sustained contraction can be produced in isolated systemic vessels in response to severe hypoxia/anoxia, but the pathophysiological

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significance of this is unclear. Thus the sustained vasoconstrictor response to *moderate* hypoxia is a property of pulmonary vessels and the contractile cells therein that distinguishes them from vessels in the systemic circulation. It follows that elucidation of the biochemical basis of HPV may reveal processes that can be exploited for the selective pharmacological manipulation of pulmonary vascular tone.

## Candidate vasoactive factors and HPV

Little is known about the mechanisms mediating HPV. One hypothesis that has been studied extensively is that hypoxia alters the balance in activity between locally-produced vasoconstrictors and vasodilators. In the search for humoral mediators many vasoactive substances have been considered as candidates. We have focused our attention on two potent vasoconstrictors, endothelin and angiotensin II, and two vasodilators, nitric oxide and the recently identified novel peptide, adrenomedullin.

Our studies have employed the isolated perfused rat lung. Briefly, rats are anaesthetized and the trachea cannulated. The lungs are left *in situ* and ventilated with 5%$CO_2$ in air at a constant rate (32 breaths $min^{-1}$) to a maximum end-expiratory pressure of 4 mmHg, giving a tidal volume of 5–7 ml. The right ventricle and left atrium are cannulated. Blood from donor (in-house Wistar) control rats is heparinised and used to perfuse the lungs via the pulmonary artery (PA); blood is returned to a reservoir via a left atrial cannula. PA pressure is measured via an indwelling pressure transducer. The perfusion blood flow rate is kept constant at 18 ml $min^{-1}$, giving a PA pressure comparable with that measured *in vivo* ($\sim$15 mmHg). Acute alveolar hypoxia is produced by changing the ventilation gas to a 2%$O_2$, 5%$CO_2$, 93%$N_2$ mixture. Since the perfusion rate is held constant, the rise in PA pressure reflects an increase in vascular tone–HPV.

Angiotensin II levels are reported to increase transiently on exposure to hypoxia [15]. Bolus administration of the peptide (0.1–10 μg) produces dose-dependent elevations in pulmonary artery pressure in the isolated perfused rat lung. This effect is blocked by angiotensin type 1 ($AT_1$, e.g. losartan) but not $AT_2$ (e.g. PD123319) receptor antagonists. Competitive radioligand binding studies using isolated lung membranes have confirmed that $AT_1$ is the major angiotensin II receptor subtype present in the rat lung and the density of angiotensin II binding sites increases in response to prolonged normobaric hypoxia (10%$O_2$). However, neither $AT_1$ nor $AT_2$ receptor antagonists influence the pressor response to acute hypoxia in the isolated lung preparation.

We and others have found that lung endothelin-1 levels are elevated two- to threefold in the first 24 to 48 h after exposure to hypoxia [16]. Both endothelin type A ($ET_A$) and $ET_B$ receptors are present in rat lung and expression of the $ET_A$ (but not $ET_B$) subtype has been found to increase during short-term (48 h) exposure to hypoxia (10%$O_2$) [17]. $ET_A$ receptors are thought to predominate in large pulmonary arteries and in veins, and $ET_B$ in small pulmonary arteries [18]. Nonetheless, continuous treatment with BQ123 (an $ET_A$ antagonist) as well as bosentan (a mixed $ET_A$ and $ET_B$ antagonist) has been reported to attenuate the rise in pulmonary artery pressure and structural remodelling produced by chronic hypoxia in the rat [19, 20]. Moreover, one report suggests that blockade of $ET_A$ receptors inhibits completely the acute pressor response to normobaric hypoxia (10%$O_2$) in the pulmonary circulation of conscious rats [21]. This is an important observation but needs to be repeated with other $ET_A$ receptor antagonists and in other experimental preparations before the definitive role of endothelin in HPV is established.

The nitric oxide precursor L-arginine and analogues of this amino acid have been used by several groups to explore the role of nitric oxide in HPV [22–24]. There is a compelling body of evidence that nitric oxide can modulate HPV. Infusions of *N*-monomethyl-L-arginine to inhibit nitric oxide synthesis increase basal pulmonary vascular tone and enhance the pressor response to hypoxia; conversely, concomitant infusion of L-arginine reduces HPV (Figure 1a and b). Continuous administration of NO by inhalation has been shown to reduce the rise in pulmonary artery pressure and vascular

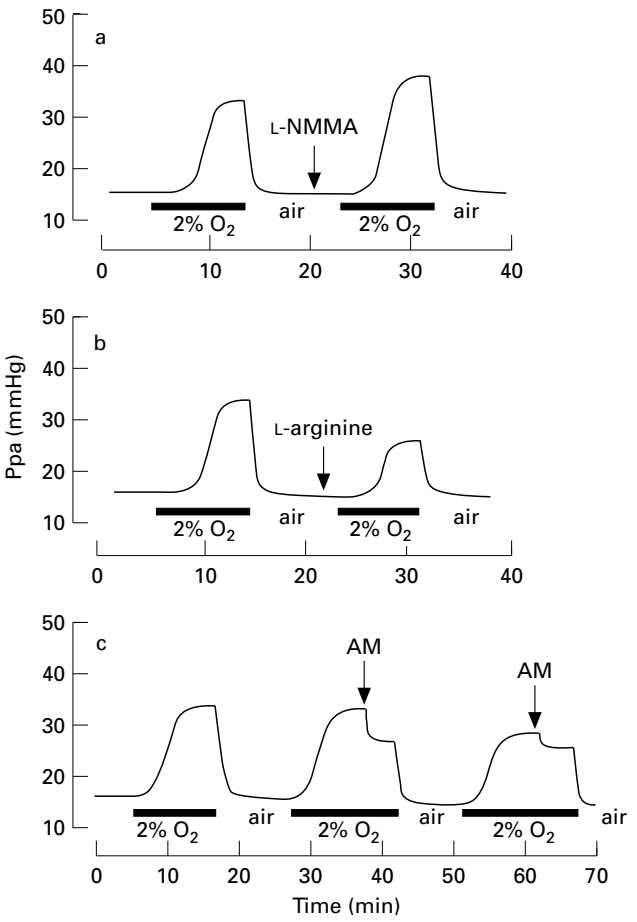


**Figure 1** Rise in pulmonary artery pressure (Ppa) in the isolated perfused rat lung during ventilation with 2%$O_2$ (solid bars) and the effect of (a) bolus dministration of *N*-monomethyl-L-arginine (L-NMMA; reservoir concentration 30 μM), (b) bolus administration of L-arginine (reservoir concentration 1 mM) and (c) adrenomedullin (AM; 2 nmol).

remodelling associated with chronic exposure to hypoxia [25]. However, the role of suppression of nitric oxide production in the mediation of HPV is still uncertain. Specifically, hypoxia has been reported to inhibit nitric oxide synthase activity in tissues other than the lung and so does not appear to provide the biochemical basis for the differential effects of low oxygen tension on systemic and pulmonary vessels. Moreover, while some groups have evidence for impaired nitric oxide production in the lungs of rats [26] kept in a chronic hypoxic environment, we and others have found that synthesis is preserved or possible increased [23, 24].

We have confirmed that the lung is an important site of synthesis of adrenomedullin by northern blot analysis. In addition, radioligand binding studies show that lung exhibits a high density of binding sites for the peptide compared with other tissues [27]. Using the isolated perfused lung we have shown that administration of adrenomedullin produces dose-dependent reductions in pulmonary artery pressure during acute hypoxia and reduces the pressor response to a subsequent hypoxic challenge (Figure 1c). Adrenomedullin levels (mRNA and peptide) in lungs from rats exposed to normobaric hypoxia (10% $O_2$) for periods up to 7 days do not differ from those of rats allowed to breathe normal air but we have measured a significant 73% increase in the density of [$^{125}$I]-adrenomedullin binding sites (with no change in dissociation constant) in chronically hypoxic rat lung [28]. At present no specific receptor antagonists are available to examine the effect of inhibition of adrenomedullin activity on the HPV response.

Other investigators have pursued the hypothesis that the contractile response to hypoxia is an intrinsic property of pulmonary vascular smooth muscle cells and caused by a direct effect of the stimulus on these cells. In support of this there is evidence that hypoxia can regulate $K^+$ channels [29], decrease oxidative phosphorylation [30] and alter the production of reactive oxygen species that regulate transmembrane $Ca^{2+}$ flux [31]. It remains to be established whether any of these observations can account for differences in the response to hypoxia between pulmonary and systemic vascular smooth muscle.

## Genetic differences in response to hypoxia

Rat strains have been reported to differ in their susceptibility to the cardiopulmonary effects of hypoxia. The Hilltop strain of male Sprague–Dawley rat develops severe pulmonary hypertension and right ventricular hypertrophy during 30 to 40 days exposure to hypoxia while the Madison strain of Sprague–Dawley rats tolerate these conditions with a significantly less marked cardiopulmonary response [32, 33]. Similarly, an attenuated response to hypoxia has also been recorded in Fischer 344 (F344) rats compared with the Sprague–Dawley strain [34].

Recent studies in our laboratory have compared the HPV response in F344 rats with that in Wistar-Kyoto (WKY) rats and outbred Wistars. Animals were studied at 12 weeks of age. Basal pulmonary artery and systemic blood pressure were similar in the two strains. There was, however, a marked difference between F344 and WKY rats in the pulmonary vascular response to hypoxia. Experiments using the isolated perfused lung preparation demonstrated that the rise in mean pulmonary artery pressure during successive hypoxic challenges (ventilation with 2%$O_2$ which produces a $PaO_2 \sim$ 30 mmHg) was two-fold greater in the WKY lung compared with the F344 lung (Figure 2), with no overlap in the response between the two strains. Chronic exposure of both rat strains to 10%$O_2$ in a normobaric chamber for 14 days ($PaO_2 \sim 40$ mmHg) showed that the differential response is maintained (Figure 3). One possibility we have considered is that the normal F344 strain has fewer or less muscularized pulmonary arterioles. However, this is not supported by our histological

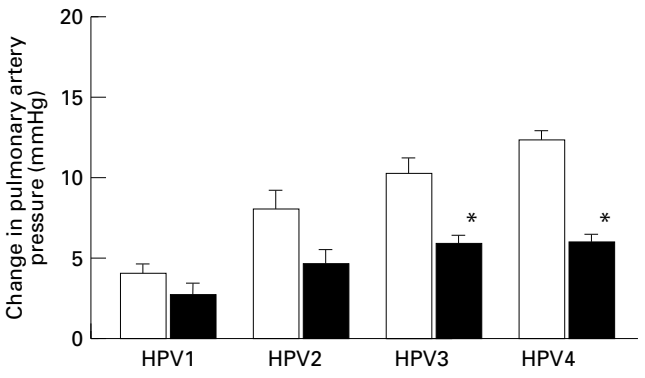


**Figure 2** The pulmonary vascular response to acute hypoxia in WKY (open bars) and F344 (closed bars) rats. Studies were conducted in the isolated perfused lung preparation. The ventilation gaseous mixture was changed to 2%$O_2$ for a period of 8 min to produce hypoxic vasoconstriction (HPV) on four successive occasions; the interval between hypoxic challenges was 10 min. There was no difference in baseline pulmonary artery pressure between the two rat strains. The rise in pulmonary artery pressure above baseline was recorded for each hypoxic challenge. The data are mean ± s.e.mean. $n = 8$ each group. *$P < 0.05$ *vs* WKY.

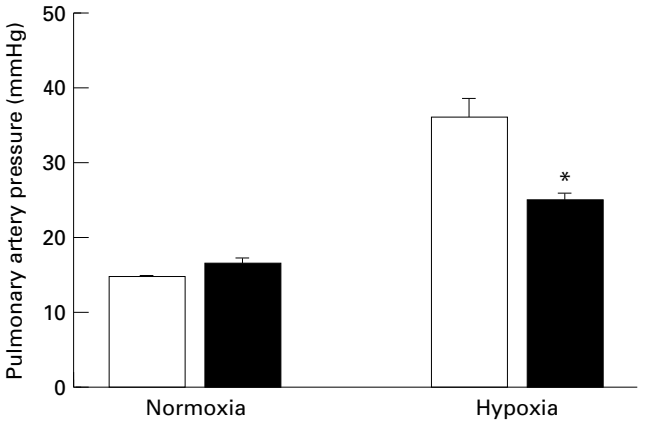


**Figure 3** Pulmonary artery pressure of normal 12 week WKY (open bars) and F344 (closed bars) rats and of a separate group of rats kept in a normobaric, hypoxic chamber ($FiO_2$ 10%) for 14 days. There was no difference in systemic blood pressure before or after the hypoxic challenge. $n = 6$ each group. *$P < 0.05$ *vs* WKY.

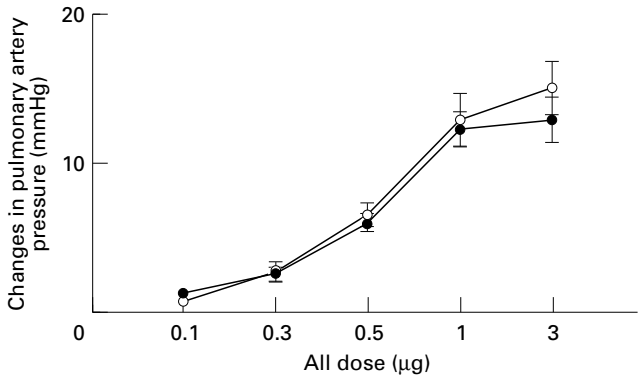


**Figure 4** Change in pulmonary artery pressure in response to increasing doses of angiotensin II (AII) in the isolated perfused lungs of WKY (open circles) and F344 (closed circles) rats. There was no difference between the two strains.

studies of lungs from the two strains. Moreover, the pulmonary circulation of the two strains showed the same pressor response to angiotensin II (Figure 4). It is unlikely that the blunted HPV is due to a generalised alteration of sensing of the $O_2$ tension because there was no difference in the final haematocrit attained by the two strains after 14 days hypoxia (WKY from $44 \pm 1$ to $61 \pm 1.8\%$; F344 form $45 \pm 0.9$ to $60 \pm 2\%$). Likewise, there was no significant difference in the systemic arterial pressure responses to acute or chronic hypoxia in these two strains.

The difference in the HPV response between F344 and WKY rats is likely to be genetically determined because the trait breeds true in these inbred rat strains and the difference in HPV response persists even when the environmental conditions and the experimental stimulus are carefully controlled.

## Future work

We propose to determine the molecular genetic basis for the difference in susceptibility to HPV using genetic segregation and linkage analysis. These techniques are now well established and have been used to identify susceptibility loci for monogenic traits such as obesity in mice [35] and polygenic disorders such as hypertension [36–38] and epilepsy [39] in rodents. Over 700 microsatellite markers spaced across the rat genome are now available. The use of anonymous markers and linkage analysis complements studies examining the role of candidate factors. Unlike the candidate factor approach, however, a systematic screen of the genome makes no prior assumptions about the mechanisms of HPV and should provide new insights into factors underlying the HPV response and suggest new targets for pharmacological intervention.

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