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The effect of the nitric oxide synthase inhibitor *N*-γ-nitro-L-argine methyl ester on hypoxic pulmonary vasoconstriction

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Abstract

We studied the role of nitric oxide in the regulation of pulmonary arterial tone and hypoxic pulmonary vasoconstriction. Rat pulmonary arteries ($n = 65$, diameter = 440 ± 12 μm) were loaded to 17.5 mm Hg in a wire myograph and incubated with the nitric oxide synthase inhibitor *N*-γ-nitro-L-argine methyl ester (L-NAME; 1, 10 or 100 μM) or distilled water (50 μl) prior to preconstriction with either 100 μM prostaglandin $F_{2\alpha}$ followed by acetylcholine (0.1–100 μM) or 5 μM prostaglandin $F_{2\alpha}$ followed by hypoxia. Concentrations of L-NAME (10 and 100 μM) which attenuated acetylcholine dilatation, elevated basal tone from $0.2 \pm 0.5\%$ to $9.4 \pm 2.1\%$ ($P < 0.01$) and $18.3 \pm 3.2\%$ ($P < 0.001$), respectively, potentiated contraction to 5 μM prostaglandin $F_{2\alpha}$ from $35.9 \pm 3.1\%$ to $56.2 \pm 6.8\%$ ($P < 0.05$) and $66.4 \pm 5.8\%$ ($P < 0.001$), respectively, but had no significant effect on hypoxic pulmonary vasoconstriction. This suggests basal pulmonary nitric oxide release occurs, as well as in response to agonist-induced contraction, but not hypoxic pulmonary vasoconstriction.

Keywords: Nitric oxide (NO); Hypoxic pulmonary vasoconstriction; Pulmonary arterial tone; L-NAME; *N*-γ-nitro-L-argine methyl ester; Prostaglandin $F_{2\alpha}$

1. Introduction

The endogenous vasodilator nitric oxide plays a key role in the pulmonary circulation, being involved in its development and maintenance, and playing an integral role in its transition from the fetal to the adult state. Nitric oxide is thought to be involved in the establishment of basal pulmonary arterial tone since, even at early stages of development, the enzyme endothelial nitric oxide synthase is detected in the lung (Halbower et al., 1994), and nitric oxide antagonists further increase the high pulmonary vascular resistance of the fetal pulmonary circulation (Cornfield et al., 1992). This basal release of nitric oxide is presumed to be protective against excessive constriction and hypertensive vascular remodeling, which increases the risk of sustained pulmonary hypertension at birth (Levin et al., 1978; Abman et al., 1989, 1991).

Nitric oxide is believed to play a similar role in the adult pulmonary circulation. Basal release of nitric oxide is suggested by the observation that endothelial nitric oxide synthase inhibition increases pulmonary tone, both in isolated pulmonary arteries and perfused lungs (Archer et al., 1989; Rodman et al., 1990; Persson et al., 1990; Omar and Wolin, 1992; Kantrow et al., 1997; Carville et al., 1997; Igari et al., 1998). Basal nitric oxide release is postulated to be responsible for the intrinsic low arterial tone of the pulmonary circulation. However in contrast, evidence that endothelial nitric oxide synthase inhibitors have no effect on basal pulmonary arterial tone, also exists (Robertson et al., 1990; Barer et al., 1993).

A similar disparity surrounds the role of nitric oxide as a modulatory factor in the pressor response of the pulmonary circulation to hypoxia; hypoxic pulmonary vasoconstriction, the underlying mechanism of which remains elusive. The potential role of nitric oxide as a modulatory factor in hypoxic pulmonary vasoconstriction in the adult is unclear, with two contrasting opinions expressed in the literature. Numerous groups have demonstrated that acute application of endothelial nitric oxide synthase inhibitors or nitric oxide antagonists results in an elevation of hypoxic pulmonary vasoconstriction (Brashers et al., 1988;

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Archer et al., 1989; Robertson et al., 1990; Persson et al., 1990; Demiryurek et al., 1991; Liu et al., 1991; Ogata et al., 1992; Ohe et al., 1992; Barer et al., 1993; Igari et al., 1998; Dumas et al., 1999). Such observations suggest that nitric oxide release occurs in response to hypoxia-induced contraction, as a protective mechanism which prevents excessive constriction. However alternatively it is proposed that it is an inhibition of the normal basal release of nitric oxide which occurs under hypoxic conditions, allowing a hypoxic contraction to be produced (Kovitz et al., 1993; Hoshino et al., 1994; Lee et al., 1995; Karamsetty et al., 1998; Terraz et al., 1999).

In the small vessel wire myograph (Cambustion, UK) hypoxic pulmonary vasoconstriction of rat pulmonary arteries is increased once the vessels have been primed with an agonist which acts to elevate the level of intracellular calcium (Zhang et al., 1995). Following priming with prostaglandin $F_{2\alpha}$ (5 μM), applying a hypoxic gas mixture results in a four phase response, the time course of which varies depending on the size of the pulmonary artery (Jones et al., 1999). In small preconstricted intrapulmonary arteries, hypoxic stress results in an initial small vasodilation (Phase 1), followed by a large hypoxic contraction which occurs for approximately 5 min (Phase 2) before further vasodilation (Phase 3). Eventually, a second smaller sustained hypoxic contraction develops, after approximately 1 h (Phase 4) (Fig. 1). Phase 2 is considered to be the physiologically relevant contraction occurring over the hypoxic oxygen tension range (Teng and Barer, 1995a).

The aim of the present study was to attempt to clarify the role of nitric oxide in the pulmonary circulation, both in the maintenance of its intrinsic low arterial tone, and in the regulation of the pressor response to hypoxia as well as receptor agonists such as prostaglandin $F_{2\alpha}$. A myographic study utilising isolated rat pulmonary arteries allows separation of the different phases of hypoxic pulmonary vasoconstriction and therefore a detail examination of the role of nitric oxide in each of these phases could be undertaken. Inhibition of endothelial nitric oxide synthase was produced via *N*-γ-nitro-L-argine methyl ester (L-NAME).

2. Materials and methods

2.1. Vessel preparation

Male Wistar rats ($n = 18$, mean body weight = 337 ± 12 g) were killed by intra-peritoneal injection of sodium pentobarbitone (15 mg/100 g body weight) as approved by UK Home Office guidelines, and the lungs removed and placed in chilled physiological saline solution. Endothelial intact pulmonary arteries ($n = 65$, mean internal diameter = 440 ± 12 μm) were carefully dissected from the transitional elastic segment (Sasaki et al., 1995) and mounted on two 40 μm stainless steel wires in the jaws of an automated wire myograph (Cambustion, UK) (Rogers et al., 1992). Vessel characteristics were obtained from length-tension plots, which were stored within the myo-

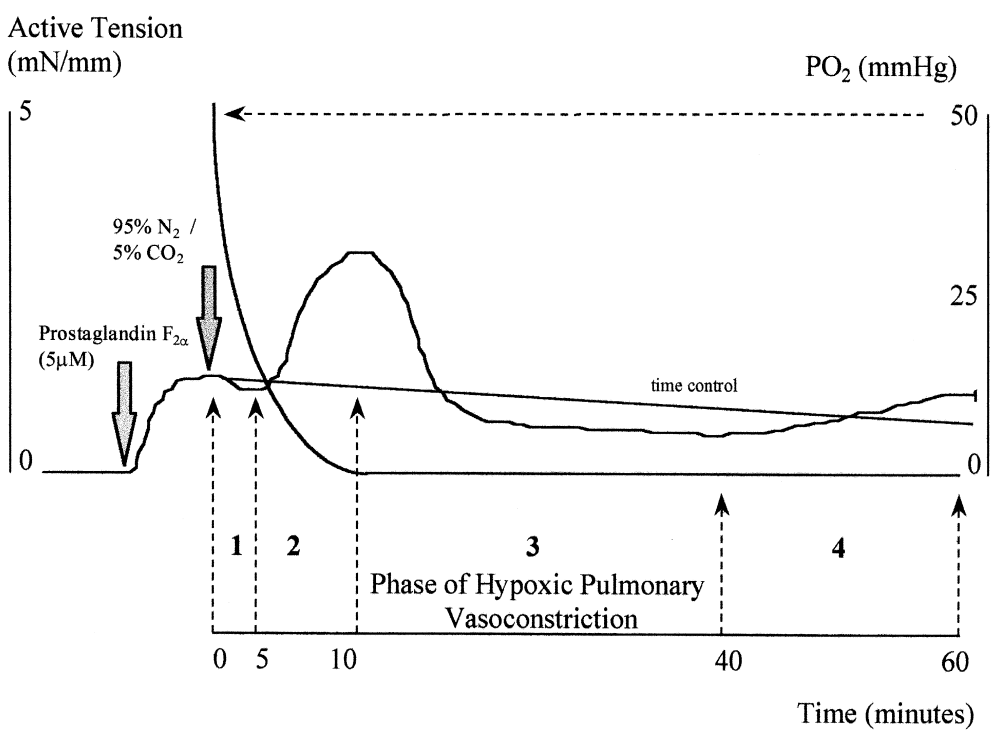

Fig. 1. Schematic representation of the four phase response of hypoxic pulmonary vasoconstriction in isolated rat pulmonary arteries mounted in a wire myograph, following priming with prostaglandin $F_{2\alpha}$ (5 μM). The time-scale (min) and corresponding PO_2 values (mm Hg) are shown in conjunction with the active tension (mN/mm).

graph software and on the basis of these, individual arteries were loaded to a resting tension equivalent to the in situ pressure of 17.5 mm Hg.

Vessels were washed by three changes of the physiological saline solution in the myograph bath, which was warmed to 37°C and bubbled continuously with 95% O_2/5% CO_2, and left to equilibrate for 1 h. Following equilibration, vessels were exposed to 80 mM potassium chloride until a maximal contraction had been produced, the physiological saline solution was then changed three times and the vessels allowed to relax back to baseline. Vessels were then re-exposed to 80 mM potassium chloride and the average of these two contractions recorded. After washing by changing the physiological saline solution three times and regaining the original baseline tension, the vessels were then exposed to 100 μM prostaglandin $F_{2\alpha}$ followed by 10 μM acetylcholine to confirm endothelial integrity. The vessels were again washed by changing the physiological saline solution three times and the baseline regained, prior to one of the following experimental protocols being undertaken.

2.2. The effect of L-NAME on the acetylcholine concentration–response curve

Pulmonary arteries ($n = 32$, mean internal diameter = 414 ± 14 μm) were exposed to either L-NAME (1, 10 or 100 μM) or vehicle (distilled water, 50 μl) for 30 min prior to preconstriction with 100 μM prostaglandin $F_{2\alpha}$. Once a maximal contraction was produced to 100 μM prostaglandin $F_{2\alpha}$, acetylcholine (0.1–100 μM) was added cumulatively to produce a concentration–response curve. Values of E_{max} and pEC_{50} were recorded as measures of activity and potency respectively. Dilation to acetylcholine was expressed as percentage relaxation of the initial 100 μM prostaglandin $F_{2\alpha}$ preconstriction.

2.3. The effect of L-NAME on basal tone and contractions to prostaglandin $F_{2\alpha}$ and hypoxic pulmonary vasoconstriction

Pulmonary arteries ($n = 33$, mean internal diameter = 466 ± 19 μm) were exposed to either L-NAME (1, 10 or 100 μM) or vehicle (distilled water, 50 μl) for 30 min prior to preconstriction with 5 μM prostaglandin $F_{2\alpha}$. Once a maximal contraction was produced to 5 μM prostaglandin $F_{2\alpha}$ the myograph bath was sealed and the active gas mixture changed to 95% N_2/5% CO_2. The vessels were left under these conditions until all four phases of hypoxic pulmonary vasoconstriction were produced. The individual phases of the hypoxic response, contraction to prostaglandin $F_{2\alpha}$ (5 μM), and the change in basal tone following L-NAME addition, were standardised via expression as a percentage of mean of the two initial 80 mM potassium chloride contractions.

2.4. Solutions and drugs

Physiological saline solution 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 μM EDTA dissolved in distilled water. All physiological saline solution reagents were obtained from Sigma, UK.

Prostaglandin $F_{2\alpha}$ was obtained from the Royal Hallamshire Hospital pharmacy, Sheffield, UK as a stock solution. L-NAME and acetylcholine were obtained from Sigma, UK and diluted to the required concentration in distilled water.

2.5. Statistical analysis

Values are expressed as mean ± standard error of the mean and were compared using the Mann–Whitney–Wilcoxon test, Kruskal Wallis non-parametric analysis of variance (ANOVA), parametric ANOVA, or by students unpaired *t*-test where appropriate. Significance was assumed with values of $P < 0.05$.

3. Results

No significant differences in vessel diameter or responsiveness were seen between any group of vessel studied. Mean pulmonary artery internal diameter was 440 ± 12 μm, and mean contraction to 80 mM potassium chloride was 3.33 ± 0.22 mN/mm. Mean contraction to 100 μM prostaglandin $F_{2\alpha}$ was 2.51 ± 0.22 mN/mm and mean dilation (occurring in all vessels) to 10 μM acetylcholine of -1.15 ± 0.06 mN/mm confirmed endothelial integrity.

3.1. The effect of L-NAME on the acetylcholine concentration–response curve

Exposure to L-NAME (1, 10 or 100 μM) for 30 min produced a concentration-dependent reduction in pulmonary arterial dilation to acetylcholine (0.1–100 μM) (Fig. 2).

Acetylcholine elicited marked pulmonary arterial vasodilation in vehicle (distilled water)-treated vessels producing an E_{max} at 100 μM of $57.9 \pm 6.6\%$ and a pEC_{50} of 5.97 ± 0.07 M. Despite some inhibition of the acetylcholine concentration–response curve occurring in vessels incubated with 1 μM L-NAME (Fig. 2), no significant effect on either E_{max} or pEC_{50} were seen; values being $38.6 \pm 7.7\%$ ($P > 0.05$ Mann–Whitney–Wilcoxon test) and 5.80 ± 0.05 M ($P > 0.05$ Student's unpaired *t*-test), respectively.

However, significant reductions in both E_{max} and pEC_{50} of the acetylcholine concentration–response curve were seen in vessels incubated with either 10 or 100 μM L-NAME. In vessels exposed to 10 μM L-NAME E_{max} and pEC_{50} were $23.1 \pm 2.6\%$ ($P < 0.001$ Mann–Whit-

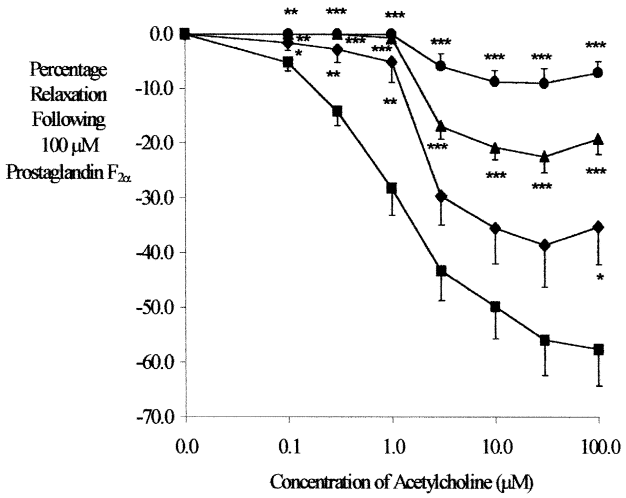

Fig. 2. Vasodilation to acetylcholine (0.1–100 μM) of isolated rat pulmonary arteries preconstricted with prostaglandin $F_{2\alpha}$ (100 μM) in the absence and presence of the endothelial nitric oxide synthase inhibitor L-NAME. ♦ — dilation to acetylcholine following 30 min incubation with L-NAME (1 μM). ▲ — dilation to acetylcholine following 30 min incubation with L-NAME (10 μM). ● — dilation to acetylcholine following 30 min incubation with L-NAME (100 μM). ■ — dilation to acetylcholine following 30 min incubation with vehicle (distilled water-50 μl). * — represents significance of $P < 0.05$. ** — represents significance of $P < 0.01$. *** — represents significance of $P < 0.001$ from vehicle treated group via Mann–Whitney–Wilcoxon test.

ney–Wilcoxon test) and 5.71 ± 0.03 M ($P < 0.01$ Student's unpaired *t*-test), respectively. In vessels exposed to 100 μM L-NAME E_{max} and pEC_{50} were $10.5 \pm 2.4\%$ ($P < 0.001$ Mann–Whitney–Wilcoxon test) and 5.38 ± 0.11 M ($P < 0.001$ Student's unpaired *t*-test), respectively.

3.2. The effect of L-NAME on basal tone

Incubation with L-NAME for 30 min (1, 10 or 100 μM) produced an increase in pulmonary arterial tone at all three concentrations.

Thirty minutes exposure to vehicle (distilled water) produced an increase in basal tone of $0.2 \pm 0.5\%$. In vessels exposed to 1, 10 or 100 μM L-NAME this increased to $9.4 \pm 3.4\%$ ($P < 0.05$ Mann–Whitney–Wilcoxon test), 9.4 ± 2.1 ($P < 0.01$ Mann–Whitney–Wilcoxon test) and $18.3 \pm 3.2\%$ ($P < 0.001$ Mann–Whitney–Wilcoxon test), respectively. The magnitude of the contraction to each concentration of L-NAME was not significantly different from each other ($P > 0.1$ Kruskal Wallis non-parametric ANOVA), although significance from control values increased at each concentration.

3.3. The effect of L-NAME on contractions to $PGF_{2\alpha}$ and hypoxic pulmonary vasoconstriction

Thirty minutes exposure to L-NAME (1, 10 or 100 μM) produced a concentration-dependent potentiation of contraction to 5 μM prostaglandin $F_{2\alpha}$ compared to vehicle (distilled water)-treated vessels, but had no significant effect upon any of the four phases of hypoxic pulmonary vasoconstriction (Fig. 3).

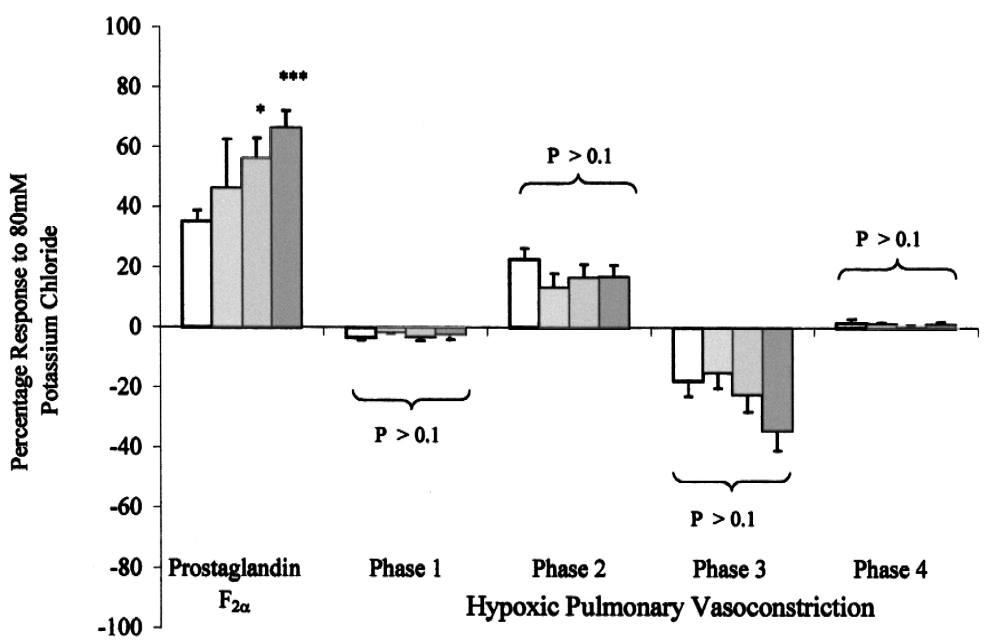

Fig. 3. The four phase response of isolated rat pulmonary arteries to hypoxia following preconstriction with prostaglandin $F_{2\alpha}$ (5 μM) post 30 min exposure to the endothelial nitric oxide synthase inhibitor L-NAME (1, 10 or 100 μM) or vehicle (distilled water, 50 μl). Responses to 5 μM prostaglandin $F_{2\alpha}$, Phase 1, Phase 2, Phase 3 and Phase 4 of hypoxic pulmonary vasoconstriction post exposure to L-NAME or vehicle are as follows: □ — post 30 min exposure to vehicle (distilled water, 50 μl). □ — post 30 min exposure to L-NAME (1 μM). □ — post 30 min exposure to L-NAME (10 μM). ■ — post 30 min exposure to L-NAME (100 μM). * — represents significance of $P < 0.05$. *** — represents significance of $P < 0.001$ from vehicle treated group via Mann–Whitney–Wilcoxon test. Statistical analysis between test groups was undertaken via Kruskal Wallis non-parametric analysis of variance as indicated by brackets. None of the phases of hypoxic pulmonary vasoconstriction were significantly different.

Mean contraction to 5 μM prostaglandin $F_{2\alpha}$ following exposure to vehicle was 35.4 ± 3.5%. In vessels treated with 1 μM L-NAME, this was increased but not significantly to 46.3 ± 16.4% ($P > 0.05$ Mann–Whitney–Wilcoxon test). However contraction to 5 μM prostaglandin $F_{2\alpha}$ was potentiated in vessels exposed to 10 and 100 μM L-NAME to 56.2 ± 6.8% ($P < 0.05$ Mann–Whitney–Wilcoxon test) and 66.4 ± 5.8% ($P < 0.001$ Mann–Whitney–Wilcoxon test), respectively.

Mean hypoxic pulmonary vasoconstriction in vehicle-treated vessels was: −3.4 ± 0.9% (Phase 1), 22.8 ± 3.5% (Phase 2), −17.7 ± 5.1% (Phase 3) and 1.7 ± 1.2% (Phase 4). In vessels treated with L-NAME (1, 10 or 100 μM) none of the phases were significantly different from the vehicle treated group ($P > 0.05$ Mann–Whitney–Wilcoxon test) or from each other ($P > 0.1$ Kruskal Wallis non-parametric ANOVA) (Fig. 3).

4. Discussion

This work has demonstrated that acute exposure to the endothelial nitric oxide synthase inhibitor L-NAME, at concentrations at which dilation to acetylcholine is markedly attenuated, has no significant effect upon hypoxic pulmonary vasoconstriction of isolated rat pulmonary arteries. Evidence does exist for a basal release of nitric oxide in these vessels since exposure to L-NAME resulted in a significant elevation in basal tone compared to vehicle. Furthermore, acute exposure to L-NAME also potentiated contractions to prostaglandin $F_{2\alpha}$ (5 μM) suggesting that nitric oxide is normally released in response to agonist-mediated pulmonary vasoconstriction.

Endothelial derived relaxing factor was first described by Furchgott and Zawadzki (1980), and identified as nitric oxide seven years later (Palmer et al., 1987). Endothelial derived relaxing factor is synthesised in endothelial cells by the enzyme endothelial nitric oxide synthase, from where it is released, diffusing into smooth muscle cells and activating the enzyme guanylate cyclase, thus increasing cellular levels of the dilatory second messenger cyclic guanosine monophosphate. Nitric oxide is long recognised as a modulatory factor in the pulmonary circulation, although its precise role remains the subject of current debate.

Nitric oxide is thought to be involved in the establishment of basal tone in the pulmonary circulation since nitric oxide antagonists elevate pulmonary arterial tone (Archer et al., 1989; Rodman et al., 1990; Persson et al., 1990; Omar and Wolin, 1992; Kantrow et al., 1997; Carville et al., 1997; Igari et al., 1998). However, evidence also exists that endothelial nitric oxide synthase inhibitors have no effect on basal pulmonary arterial tone (Robertson et al., 1990; Barer et al., 1993).

Nitric oxide may also modulate pulmonary vasoconstriction. Nitric oxide release is proposed to occur in response to constriction to endogenous agents such as endothelin-1 (Barer et al., 1993) and angiotensin II (Liu et al., 1991), since pulmonary vasoconstriction induced by these agents is elevated in the presence of endothelial nitric oxide synthase inhibitors. However, a modulatory role in the control of hypoxic pulmonary vasoconstriction is split between two contrasting hypotheses.

Numerous groups have demonstrated that inhibition of nitric oxide results in an elevation of hypoxic pulmonary vasoconstriction. Endothelial nitric oxide synthase inhibitors have been shown to potentiate hypoxic pulmonary vasoconstriction in a variety of preparations, including isolated pulmonary arteries (Archer et al., 1989; Demiryurek et al., 1991; Ogata et al., 1992; Ohe et al., 1992), isolated perfused lungs (Archer et al., 1989; Robertson et al., 1990; Liu et al., 1991; Barer et al., 1993; Igari et al., 1998; Dumas et al., 1999) and ventilated open-chest animals (Persson et al., 1990). Other mechanisms by which nitric oxide levels are lowered have also been shown to potentiate hypoxic pulmonary vasoconstriction. These include endothelial destruction, addition of the soluble guanylate cyclase inhibitor methylene blue (Ogata et al., 1992; Ohe et al., 1992), the nitric oxide antagonists eicosatetraynoic acid, nordihydroguaiaretic acid and hydroquinone (Brashers et al., 1988), and also addition of oxyhaemoglobin, which inactivates nitric oxide (Ogata et al., 1992). These reports also span a variety of species including rat, rabbit, sheep, pig and human. Such observations suggest that, as with pharmacological agents, nitric oxide release occurs in response to hypoxia-induced contraction as a protective mechanism which prevents excessive constriction.

However, an alternative hypothesis has been proposed. In contrast to the above reports continuous nitric oxide release is postulated to contribute to the inherent low pulmonary arterial tone which is inhibited under hypoxic conditions. Consequently an elevation of pulmonary artery pressure ensues, being expressed as a hypoxic contraction (Kovitz et al., 1993; Hoshino et al., 1994; Lee et al., 1995; Karamsetty et al., 1998; Terraz et al., 1999). This hypothesis is supported by the reports of endothelial nitric oxide synthase inhibitors elevating basal tone (Archer et al., 1989; Rodman et al., 1990; Persson et al., 1990; Omar and Wolin, 1992; Kantrow et al., 1997; Carville et al., 1997; Igari et al., 1998) and also by observations that exhaled nitric oxide (Grimminger et al., 1995) and the synthesis of oxidation products of nitric oxide (Kantrow et al., 1997), are reduced under hypoxia.

This hypothesis was proposed on the basis that endothelial nitric oxide synthase inhibitors reduce rather than potentiate hypoxic pulmonary vasoconstriction (Kovitz et al., 1993; Hoshino et al., 1994; Lee et al., 1995; Karamsetty et al., 1998; Terraz et al., 1999). However if nitric oxide release is indeed reduced under hypoxia, then endothelial nitric oxide synthase inhibitors would not be expected to inhibit hypoxic pulmonary vasoconstriction.

Similar findings are described by Greenberg and Kishiyama (1993), Teng and Barer (1995b) and also Omar and Wolin (1992), who suggest that nitric oxide is in fact released from endothelial cells in response to the hypoxia-induced constriction, but under hypoxic conditions the production or activity of nitric oxide is altered. Since oxygen is required for the generation of nitric oxide from L-arginine, an alternative active species could be produced which modulates hypoxic pulmonary vasoconstriction in this way. Consequently, endothelial nitric oxide synthase inhibitors would now attenuate rather than potentiate hypoxic pulmonary vasoconstriction. Indirect evidence also suggests that nitric oxide activity is lower under hypoxic conditions; pulmonary vasodilation to acetylcholine, which occurs via nitric oxide release, has been shown to be reduced under hypoxic conditions (Johns et al., 1989; Demiryurek et al., 1991).

The data from the present study show that no significant alteration; neither increase nor decrease was seen in any phase of hypoxic pulmonary vasoconstriction, be it constrictor or dilator, following treatment with the endothelial nitric oxide synthase inhibitor L-NAME (Fig. 3). This occurred utilising concentrations of L-NAME high enough to markedly inhibit dilation to acetylcholine (Fig. 2). L-NAME did however result in a significant elevation in basal tone and potentiate contractions to prostaglandin $F_{2\alpha}$ (Fig. 3). These observations suggest that release of nitric oxide is not a modulatory control for hypoxia-mediated contractions, and that in contrast to Greenberg and Kishiyama (1993), nitric oxide release does not mediate any of the dilatory phases of the response of isolated rat pulmonary arteries to hypoxia. However, a basal release of nitric oxide is suggested by the observed elevation in basal tone by L-NAME.

These findings agree with earlier reports of a basal nitric oxide release contributing to the low arterial tone of the pulmonary circulation (Archer et al., 1989; Rodman et al., 1990; Persson et al., 1990; Omar and Wolin, 1992; Kantrow et al., 1997; Carville et al., 1997; Igari et al., 1998). However, they do not agree with previous observations of alterations in hypoxic pulmonary vasoconstriction following endothelial nitric oxide synthase inhibition.

Variance in such results may depend upon the species and preparation used. The reports of endothelial nitric oxide synthase inhibition potentiating hypoxic pulmonary vasoconstriction occur primarily in isolated perfused lungs or the intact animal (Brashers et al., 1988; Archer et al., 1989; Robertson et al., 1990; Persson et al., 1990; Liu et al., 1991; Barer et al., 1993; Igari et al., 1998; Dumas et al., 1999). The only report of hypoxic pulmonary vasoconstriction of isolated rat pulmonary arteries being potentiated following endothelial nitric oxide synthase inhibition utilised main extra-lobar pulmonary arteries (Archer et al., 1989). However, other reports utilising similar vessels to those in this study show endothelial nitric oxide synthase inhibition to reduce hypoxic pulmonary vasoconstriction (Greenberg and Kishiyama, 1993; Teng and Barer, 1995b; Lee et al., 1995; Karamsetty et al., 1998; Terraz et al., 1999). Clearly, the contrast between these reports and the findings of the present study cannot be explained by species or preparation variation. However, these authors are also unable to provide an explanation for their findings, suggesting that reduction in hypoxic pulmonary vasoconstriction following endothelial nitric oxide synthase inhibition is due somehow to reduced nitric oxide release. Only Karamsetty et al. (1998) postulate as to how the reduced production of a vasodilatory agent can reduce, rather than potentiate a contraction, proposing an alteration in potassium channel activity.

Consequently, it would appear that in preparations where the whole lung is intact nitric oxide release is triggered by hypoxic pulmonary vasoconstriction. However in the individual arteries, the level at which hypoxia is sensed (Zhang et al., 1997) this is clearly not the case and hypoxic pulmonary vasoconstriction may indeed be due to production of an alternative reactive species as previously suggested.

Our data also provide evidence for a modulatory role of nitric oxide in agonist induced pulmonary constriction, as demonstrated by contractions to prostaglandin $F_{2\alpha}$ (5 μM) being potentiated by L-NAME. This agrees with the work of Liu et al. (1991) and Barer et al. (1993) who showed that endothelial nitric oxide synthase inhibition elevated contractions to angiotensin II and endothelin-1, respectively. Such observations also highlight the difference in regulation of hypoxia-induced pulmonary contractions and agonist-mediated contraction. It would appear that nitric oxide is released in response to agonist vasoconstriction but not due to hypoxic pulmonary vasoconstriction.

In summary, this work has shown that acute exposure to L-NAME has no effect on any phase of hypoxic pulmonary vasoconstriction but does result in a significant elevation of basal vessel tension. The two highest concentrations of L-NAME also potentiated contraction to prostaglandin $F_{2\alpha}$. This suggests that a basal release of nitric oxide occurs in the pulmonary circulation, as well as in response to agonist-induced contraction to agents such as prostaglandin $F_{2\alpha}$, but not hypoxia. Inhibition of this basal release of nitric oxide may contribute to the hypoxic contraction of the pulmonary circulation in vivo but not in isolated vessels.

Acknowledgements

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