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ENDOTHELIAL CONTROL OF THE PULMONARY CIRCULATION IN NORMAL AND CHRONICALLY HYPOXIC RATS

BY GWENDA BARER, CELIA EMERY, ALAISDAIR STEWART, DENISE BEE AND PETER HOWARD

From the Department of Medicine and Pharmacology, University of Sheffield, Royal Hallamshire Hospital, Sheffield S10 2JF

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SUMMARY

1. The effect of blockade of nitric oxide synthesis in pulmonary endothelium by two L-arginine analogues was tested in isolated blood-perfused lungs of normal rats and rats exposed chronically to 10% O_2.

2. In both groups of rats the analogues (*N*-monomethyl-L-arginine (L-NMMA) and *N*-nitro-L-arginine methyl ester (L-NAME)) enhanced hypoxic vasoconstriction. In normal rats, with rare exceptions, these analogues had little or no effect on pulmonary artery pressure (P_{pa}) at constant blood flow during normoxia. However, chronically hypoxic rats have pulmonary hypertension and in these rats the analogues always raised P_{pa}; the rise in P_{pa} after L-NMMA but not L-NAME could be partially reversed by L-arginine. L-NAME was more potent than L-NMMA.

3. To see whether the difference between rat groups was due to the high P_{pa} in chronically hypoxic rats, in *control* rats we raised P_{pa} passively by lung inflation to values higher than found in chronically hypoxic rats. L-NAME did not alter the effects of lung inflation on P_{pa}.

4. P_{pa} was also raised passively by plotting pressure–flow lines up to high flow rates; the lines were changed minimally by both analogues in control rats but in chronically hypoxic rats the lines were raised to higher pressures and steepened substantially.

5. In control rats, during vasoconstriction caused by hypoxia, endothelin 1 and almitrine, L-NAME caused further rises in pressure. We conclude that a stimulus for nitric oxide release in control rats is the narrowing of vessels caused by vasoconstriction rather than passive increases in intravascular pressure.

6. In chronically hypoxic rats arterioles are narrowed by growth of new muscle and there is some muscle tone even in normoxia. Thus narrowing of the vascular lumen is the stimulus common to both groups of rats which leads to nitric oxide synthesis and attenuation of P_{pa} by a negative feedback process. Narrowing is associated with a large increase in shear stress due to two factors; the pressure drop along a vessel segment is increased and the surface area of the lining of the affected segment is decreased.

7. Atrial natriuretic peptide caused dose-dependent pulmonary vasodilation in

both rat groups but had a greater effect in chronically hypoxic rats. The action persisted and was enhanced after blockade of NO synthesis.

INTRODUCTION

Moncada and colleagues have evidence that the endothelial-derived relaxant factor, nitric oxide (NO), is continuously released in the systemic circulation and attenuates the arterial pressure (reviewed by Moncada, Palmer & Higgs, 1991). Nitric oxide is formed from L-arginine by NO synthase. The role of NO in the normal pulmonary circulation has not been fully explored. Blockade of NO synthesis by L-arginine analogues in normal rats was shown to enhance hypoxic pulmonary vasoconstriction but to have no effect on normoxic pulmonary artery pressure (Robertson, Warren & Nye, 1990*a*). The pulmonary vasculature is normally in a very low state of tone; its smooth muscle is inactive and the action of dilator substances cannot be demonstrated unless tone is first raised. The question has been raised before as to whether this dilated state is actively or passively maintained (Weir, 1978). We considered whether dilatation was maintained by activity of NO and whether this substance might attenuate the pressor effects of natural pulmonary vasoconstrictor stimuli. In order to explore the role of NO in both the normal and hypertensive pulmonary circulation, we used the two arginine analogues L-NMMA and L-NAME (*N*-monomethyl-L-arginine and *N*-nitro-L-arginine methyl ester) to block its synthesis in normal and chronically hypoxic rats. Rats exposed for several weeks to a low O_2 environment develop pulmonary hypertension and abnormally high pulmonary vascular tone. In preliminary results (Barer, Emery & Bee 1990*a*, *b*, 1991), we found that NO synthesis blockade had greater effects on pulmonary artery pressure in chronically hypoxic than control rats. In this work we have extended these observations and examined possible causes of the difference between these two rat groups with respect to stimuli which may cause NO release.

METHODS

Male Wistar SPF rats (A. Tuck & Son) were used in all experiments where comparisons were made between control (C) and chronically hypoxic (CH) rats. The local University strain of Wistar rats was used for some tests on C rats; 122 rats were used in all. Rats were placed in a normobaric environmental chamber maintained at 10% O_2 when 33 days old; they were kept there for 2–3 weeks and then compared with littermate controls kept in the same room in air, as previously described (Emery, Bee & Barer, 1981). Tests were made on CH rats within a few hours of removal from the chamber (maximum interval 5 h) and C rats were tested at comparable ages.

Isolated perfused lungs

After pentobarbitone anaesthesia (60 mg kg^{-1} I.P.) the chest was opened and the lungs were perfused *in situ* with blood of normal haematocrit (volume approximately 10 ml) taken from normal rats, at 38 °C, as previously described (Emery *et al.* 1981). The basal perfusion rate was 20 ml min^{-1} for both C and CH rats, as we had found the lung vascular volume to be similar in CH rats despite retarded body growth (Emery *et al.* 1981). pH was adjusted to 7·35–7·45 with bicarbonate. Ventilation was with air +5% CO_2 (normoxia), or 7, 5, 3 or 2% O_2+5% CO_2 (hypoxia). Pressures were measured with electromanometers (Druck) and flow with a Wyatt electromagnetic flowmeter (Wyatt, 1961); both were displayed on a pen recorder (Bryans, UK) or on the two axes of an *X–Y* recorder (Bryans, UK). In this constant flow preparation, changes in pulmonary artery pressure, P_{pa}, reflect changes in pulmonary vascular resistances. The perfusion

was started with air ventilation. When P_{pa} was stable we changed to ventilation with 2% O_2; this test was continued until P_{pa} had risen to a plateau, or, in some tests, had reached a maximum and begun to decline. The time to maximum P_{pa} varied between animals from 5–10 min. On returning to air ventilation P_{pa} fell rapidly; an interval of at least 5 min, during which a stable normoxic P_{pa} was established, was allowed before further tests were made.

Lung inflations

To examine whether high intravascular pressures could stimulate NO release, the lungs were inflated, after stopping ventilation, to 5, 10 and 15 mmHg inflation pressure by blowing air + 5% CO_2 over a water trap. This consisted of a T-piece whose vertical limb could be immersed in water to the appropriate depth.

Pressure–flow lines

Pressure–flow (P–$\dot{Q}$) lines were measured over a wide range of flow rates because high flow rates, associated with shear stress, are thought to be one stimulus for NO releases. They were measured in two ways: (1) the perfusion pump output was varied at measured flow settings; only P_{pa} was recorded and measurements were plotted graphically; (2) the flow meter was inserted into the perfusion circuit; flow was displayed on the x-axis and P_{pa} on the y-axis of the X–Y recorder. A line was drawn through the trace in its linear portion (flow > 5 ml min) and the intercept of this line on the pressure axis together with its slope were measured. In both methods about half a minute was allowed after each flow change for pressure to adapt to the new flow rate.

Drugs

Drugs were obtained from the following suppliers. L-NAME (Sigma, UK; stock solution 1 mg ml^{-1} in 0·9% NaCl); L-NMMA (Wellcome Research Laboratories, UK; stock solution 10 mg ml^{-1} in 0·9% NaCl); L-arginine (Sigma, UK). Almitrine (Servier Laboratories, France) and endothelin 1 (Sigma, UK) were given to cause pulmonary vasoconstriction. Atrial natriuretic peptide (ANP, Sigma, UK), shown to cause pulmonary vasodilatation, was given before and after NO blockade to see whether its action was dependent on NO release.

Statistics

Means and standard errors of the mean (S.E.M.) are given throughout. Means are compared by paired or unpaired t tests as appropriate and $P < 0{\cdot}05$ values are considered statistically significant.

RESULTS

Effect of L-NAME and L-NMMA on normoxic pulmonary artery pressure and on hypoxic vasoconstriction

L-NAME, first series

In nine C and ten CH rats, ventilation with 2% O_2 caused a greater rise in P_{pa} after L-NAME (100 μg in 0·1 ml added to the reservoir; final concentration approximately 10^{-5} M; Table 1). The figures quoted are for the tests immediately before and after giving L-NAME. However, we noticed that the second hypoxic challenge after L-NAME often caused a greater rise in P_{pa} than the first. Therefore, in the later experiments in this series, we waited for 10–20 min after the addition of L-NAME before repeating the hypoxic challenge. We found no rise in normoxic (baseline) P_{pa} in six C rats but a rise of $9{\cdot}2 \pm 1{\cdot}2$ mmHg in P_{pa} in seven CH rats (Fig. 1 and Table 1). Table 1 also shows that initial normoxic P_{pa} was higher in CH than C rats as in all other groups in these experiments and as reported previously (Emery *et al.* 1981).

L-NMMA

Similarly, L-NMMA (1 mg in 0·1 ml added to the reservoir; approximately 10^{-4} M) also raised the normoxic pressure $5{\cdot}4 \pm 1{\cdot}7$ mmHg in eleven CH rats but had only a

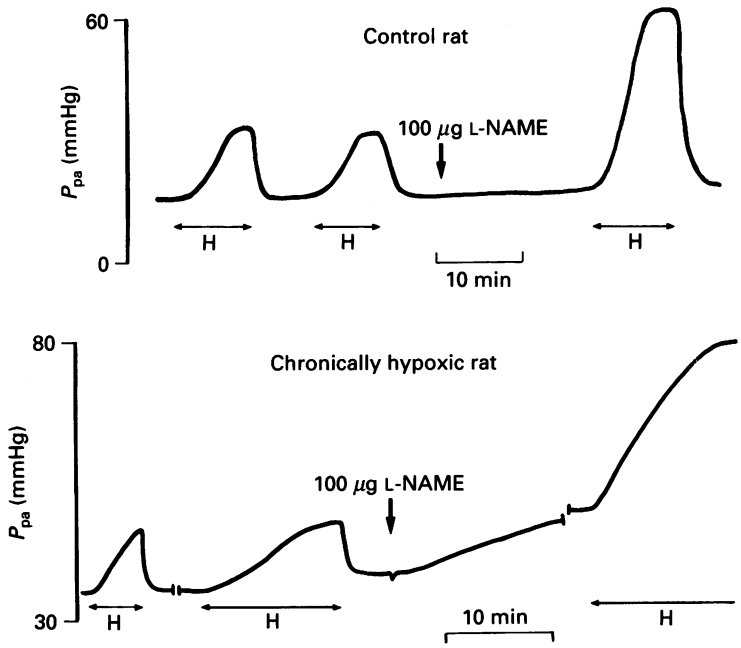

Fig. 1. Traces of pulmonary artery pressure, P_{pa}, in isolated perfused lungs of a control rat (above) and a chronically hypoxic rat (below). Ventilation changed from air + 5% CO_2 to 2% O_2 + 5% CO_2 during bars (H). In each rat, two hypoxic challenges are followed by a dose of L-NAME. This had a negligible effect on normoxic pressure in the control but caused a large rise in the chronically hypoxic rat. In both rats the subsequent hypoxic test gave an enhanced response. Break in line in lower trace indicates a few minutes.

TABLE 1. L-arginine analogues, pulmonary artery pressure, P_{pa} and hypoxic vasoconstriction

Analogues and measurements	P_{pa} (mmHg) Control rats	Chronically hypoxic rats
Initial P_{pa}	12·0±0·6 (9)	28·9±3·1 (10), $P < 0·001$
L-NAME		
ΔP_{pa} with 2% O_2		
Before blocker	+11·4±2·1 (9)	+12·2±2·1 (10)
After blocker	+27·7±5·4, $P < 0·002$	+23·2±4·1, $P < 0·02$
ΔP_{pa} in normoxia	0 (6)	+9·2±1·2 (7)
L-NMMA		
ΔP_{pa} with 2% O_2		
Before blocker	+12·2±2·3 (9)	+9·1±2·9 (7)
After blocker	+22·8±4·0, $P < 0·005$	+24·4±6·9, $P < 0·05$
ΔP_{pa} in normoxia	+1·0±0·4 (11)	+5·4±1·7 (11), $P < 0·05$

All values are means±S.E.M. with the numbers in parentheses. Initial P_{pa} values in control and chronically hypoxic rats compared by unpaired t test. The effect of L-NAME and L-NMMA on P_{pa} in normoxia and on ΔP_{pa} caused by hypoxia compared by paired t test.

trivial effect in eleven C rats ($+1{\cdot}0 \pm 0{\cdot}4$ mmHg). L-NMMA increased the effect of ventilation with 2% O_2 in both groups (see Table 1).

L-NAME second series

In further experiments L-NAME invariably raised normoxic P_{pa} in CH rats. In C rats there was usually little or no rise in P_{pa} after L-NAME during normoxia; however, sometimes small and occasionally large rises took place so that there was some overlap with the CH group. Figure 2 is a distribution curve which shows the

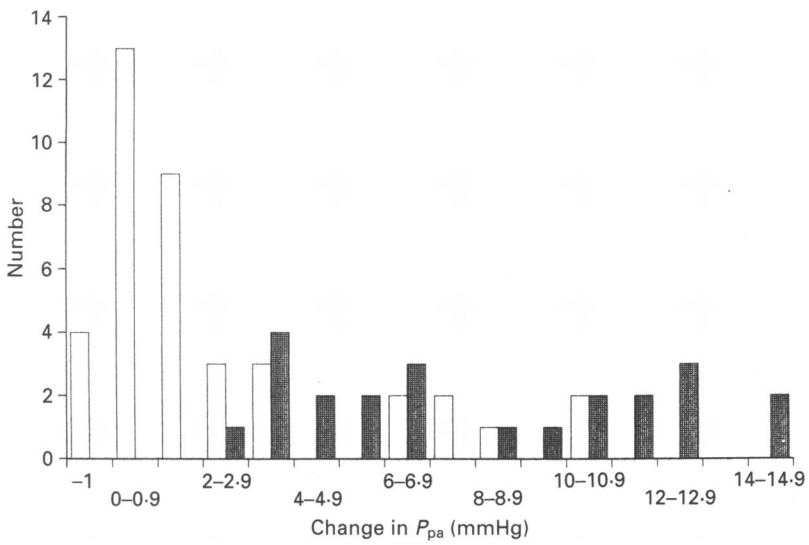

Fig. 2. Distribution curve showing the change in normoxic pulmonary artery pressure, P_{pa}, after L-NAME in control rats (□) and chronically hypoxic rats (■).

changes in P_{pa} caused by L-NAME during normoxia in all C and CH rats (series 1 and 2; series 2 includes some rats on which some other tests, described below, were made). A clear distinction is seen between the responses of the C and CH groups; there is a suggestion of two *sub*-groups in the CH rats. In the experiments described in Table 1, NO blockade took place after only two or three hypoxic tests and at a time when there had been little rise in baseline normoxic pressure. In later experiments the time from the start of the experiment had been greater and more preliminary tests had been made. We considered whether NO blockade might affect normoxic P_{pa} in C rats when there had been a rise in P_{pa} and vasoconstriction during the course of the experiment. There is usually some rise in P_{pa} in the course of a long experiment in C rats; this rise is more pronounced in CH rats (Emery *et al.* 1981). Figure 3 shows the relationship, in C rats, between the effect of L-NAME on P_{pa} and the P_{pa} just before L-NAME was given; a trend is apparent but there is much between-animal variation. Thus NO may be released in normal rats when the pressure is abnormally high.

In two out of forty-eight experiments on C rats we saw a precipitous rise in P_{pa} after addition of L-NAME during hypoxia, at the basal flow rate; this was followed

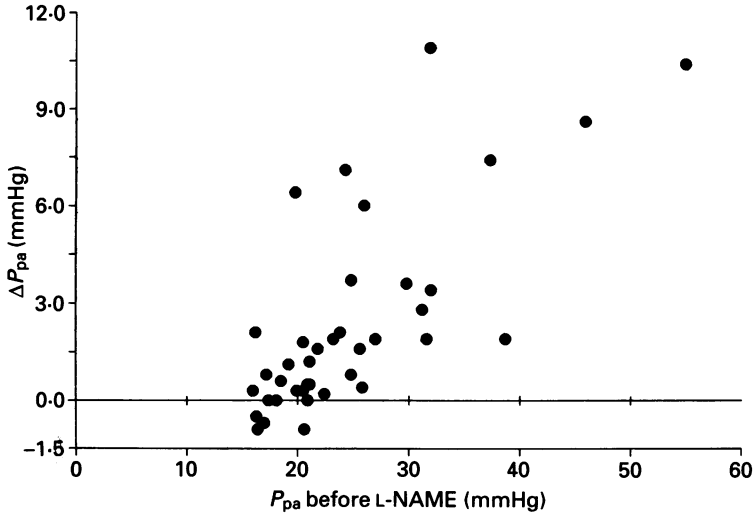

Fig. 3. Relation between the rise in normoxic pulmonary artery pressure, P_{pa}, caused by L-NAME and the pressure immediately before giving L-NAME.

by oedema. In these rats the initial P_{pa} was high and rose steeply during the experiment. Thus there are circumstances in which NO blockade may lead to instability of pressure but we cannot yet define them.

The above experiments show that L-NAME was more potent than L-NMMA in affecting hypoxic vasoconstriction and normoxic P_{pa}. If the effects are due to blockade of NO synthesis from L-arginine, it should theoretically be possible to reverse them with excess L-arginine. We were never able to modulate the rise in P_{pa} in CH rats caused by L-NAME but after L-NMMA, L-arginine reduced P_{pa} slightly ($-2{\cdot}9 \pm 0{\cdot}6$ mmHg, $n = 6$).

Effect of L-NAME on P_{pa} raised passively by lung inflation at high pressure

To cause a passive increase in intravascular pressure, we raised P_{pa} by inflation of the lung at 5, 10 and 15 mmHg pressure while maintaining a constant pulmonary blood flow. In eight C rats this caused rises in P_{pa} similar to the rises in inflation pressure and the effect was unchanged after L-NAME (Fig. 4, upper trace). The mean rise in P_{pa} caused by the 10 mmHg increase in inflation pressure (from 5 to 15 mmHg) was $8{\cdot}8 \pm 0{\cdot}5$ before and $9{\cdot}3 \pm 0{\cdot}6$ mmHg after L-NAME. By contrast, in four CH rats, as has been previously shown (Barer, Russell, Cai & Emery, 1990), the rise in P_{pa} during inflation was greater than the rise in inflation pressure and increased after L-NAME addition ($14{\cdot}7 \pm 1{\cdot}7$ before and $21{\cdot}5 \pm 1{\cdot}9$ mmHg after L-NAME; Fig. 4, lower trace).

Effect of L-NMMA and L-NAME at high flow rates. Pressure–flow lines

L-NMMA

In six C and five CH rats (these rats are included in the group shown in Table 1) P–$\dot{Q}$ lines were measured before and after L-NMMA. In CH rats the line was

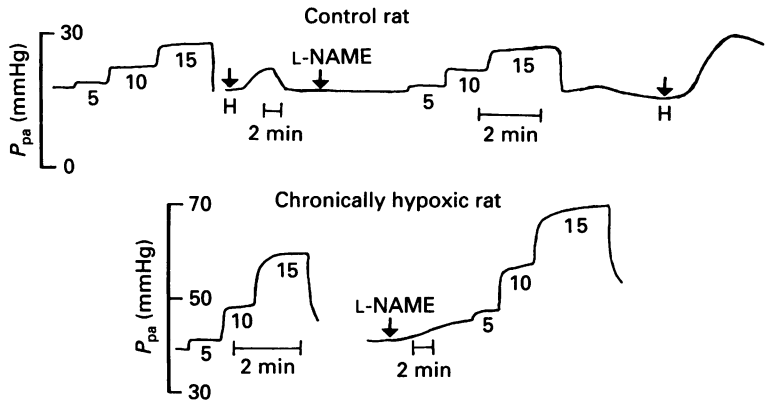

Fig. 4. Traces of the effect on pulmonary artery pressure, P_{pa}, of inflation of the lung to 5, 10 and 15 mmHg. Upper trace, in a control rat, lung inflations caused rises in P_{pa} similar to the rise in inflation pressure. The effect of inflations was not changed after L-NAME. L-NAME had no effect on normoxic pressure but enhanced the effect of hypoxia (H, 2% O_2). Lower trace, in a chronically hypoxic rat L-NAME raised P_{pa}. Inflations caused a rise in P_{pa} greater than the rise in inflation pressure and these rises in P_{pa} were greater after L-NAME. The recorder speed was greater during inflations than during intervening tests, as shown by bars.

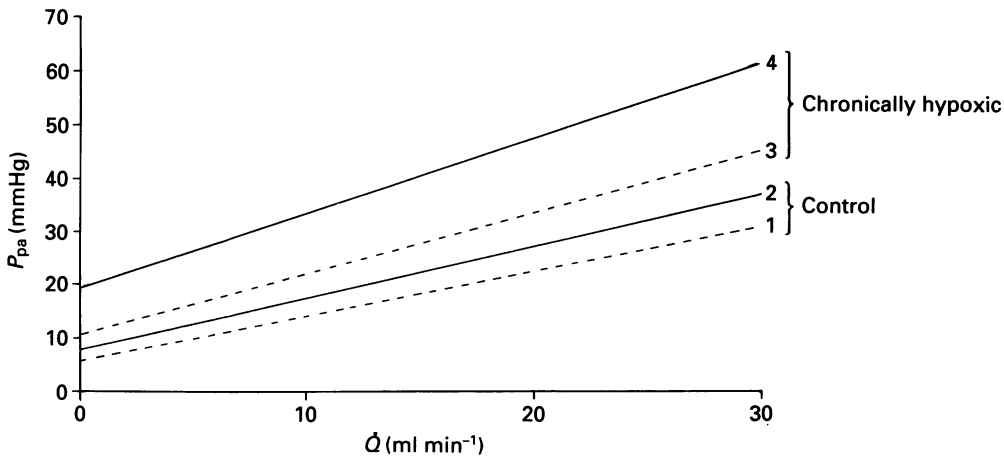

Fig. 5. Mean pressure–flow lines in six control (lines 1 and 2) and five chronically hypoxic rats (lines 3 and 4), before (– – –) and after (——) 1 mg L-NMMA.

invariably displaced to higher pressures after L-NMMA but in C rats it was superimposed or only slightly raised. Figure 5 shows the mean pressure–flow lines in the first experiment where flow was determined by pump setting. Neither the slope nor the intercept of the C lines were altered significantly after L-NAME. In CH rats the intercept ($P < 0{\cdot}05$, unpaired t test) but not the slope was significantly increased.

In a second series of experiments on six C and six CH rats (X–Y recorder method) results were similar to those of the previous group. After addition of L-NAME there was no change in the slope of the line in four C rats and a trivial upward movement of the line in two; there was an upward displacement of the line to higher flow rates

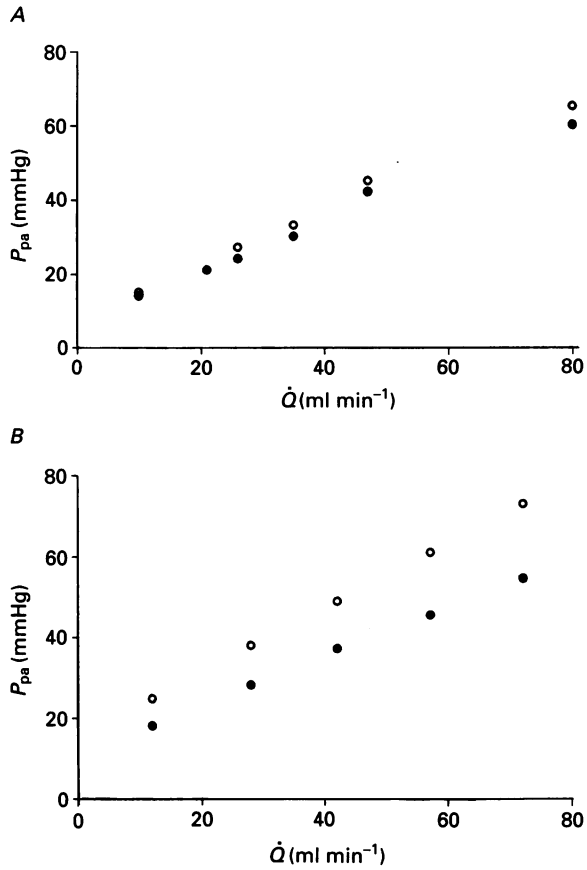

Fig. 6. Pressure–flow lines extended to a high flow rate in two control rats before (●) and after (○) L-NAME. *A*, L-NAME leads to a negligible shift (maximum flow rate 0·25 ml min^{-1} g^{-1}). *B*, there is a moderate shift but no steep increase in pressure at the high flow (maximum flow rate 0·31 ml min^{-1} g^{-1}).

in all six CH rats. Nevertheless, using both methods of measuring flow showed that an effect of NO blockade could occasionally be detected at high flow rates in *control* rats.

L-NAME

We made further P–$\dot{Q}$ measurements before and after L-NAME over a wider flow range in four C rats. In two out of four there was some shift in the line, as illustrated in Fig. 6, lower graph. In several experiments we held the high flow rate for 3–6 min but P_{pa} did not rise more than 1–2 mmHg during this period, although it was higher than 60 mmHg, a value which we would expect to lead to pulmonary oedema.

Effect of L-NAME on P_{pa} in control rats during active vasoconstriction caused by hypoxia, endothelin 1 and almitrine

In contrast to the negligible effect of L-NAME on baseline normoxic pressure in C rats, this analogue caused a further rise in pressure during hypoxic vasoconstriction

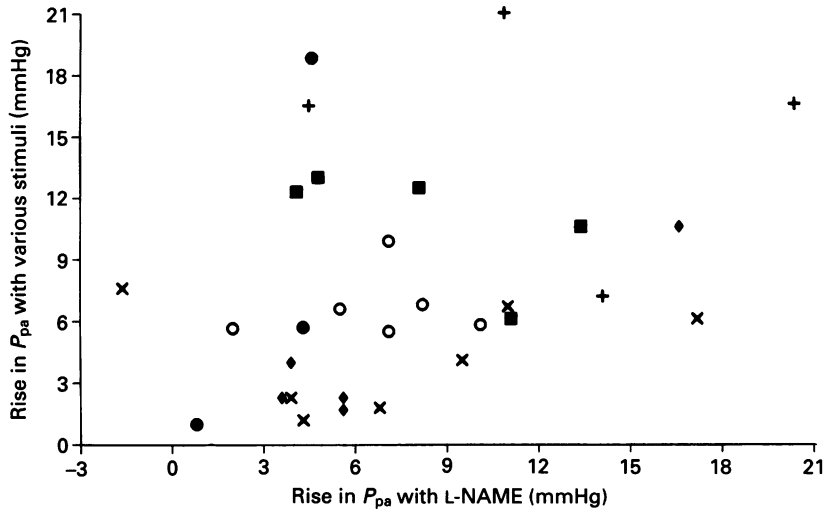

Fig. 7. Increase in pulmonary artery pressure, P_{pa}, after addition of L-NAME during vasoconstriction in control rats. The rise in pressure caused by L-NAME is plotted against the rise in pressure-caused by vasoconstriction due to hypoxia of different degrees (7 (○), 5 (◆), 3 (■) and 2% (●) O_2), endothelin 1 (100 ng (+)) and almitrine (5 μg (×)).

caused by ventilation with 7, 5, 3 and 2% O_2; mean rises in P_{pa} after L-NAME during hypoxia were: for 7% O_2, 6·7 ± 1·0 mmHg ($n = 6$); for 5%, 7·1 ± 2·2 mmHg ($n = 5$); for 3%, 8·3 ± 1·6 mmHg ($n = 5$); for 2%, 0·8, 4·6 and 4·3 mmHg ($n = 3$). The increases were variable and not clearly related to the different degrees of vasoconstriction caused by the hypoxic mixtures, as shown in Fig. 7. The mean pulmonary venous P_{O_2} caused by these mixtures was, respectively, 8·8 ± 0·3, 6·8 ± 0·2, 4·8 ± 0·2, and 3·3 ± 0·1 kPa (n = 7–12 for each mixture).

A slow but large rise in P_{pa} followed administration of 150–200 ng endothelin 1 ($n = 4$). At the height of this rise L-NAME caused a further rise in P_{pa} (12·5 ± 2·9 mmHg). These results are also shown in Fig. 7. The rise in P_{pa} caused by endothelin was usually greater than that caused by hypoxia but the further rise caused by L-NAME was within the same range as the rise found during hypoxia.

In seven experiments L-NAME was given after vasoconstriction caused by 5 μg almitrine. Almitrine caused a large pressure rise followed by a fall to a level above control which was sustained for many minutes. L-NAME was given during this plateau period and it is the plateau value that is plotted on the ordinate in Fig. 7. The mean further rise caused by L-NAME was 7·3 ± 2·1 mmHg.

Figure 7 suggests that, in all the vasoconstriction experiments considered

together, there might be some relationship between the degree of vasoconstriction, as deduced from the rise in P_{pa} it caused, and the further vasoconstriction caused by NO blockade. This may be obscured by wide between-animal variation.

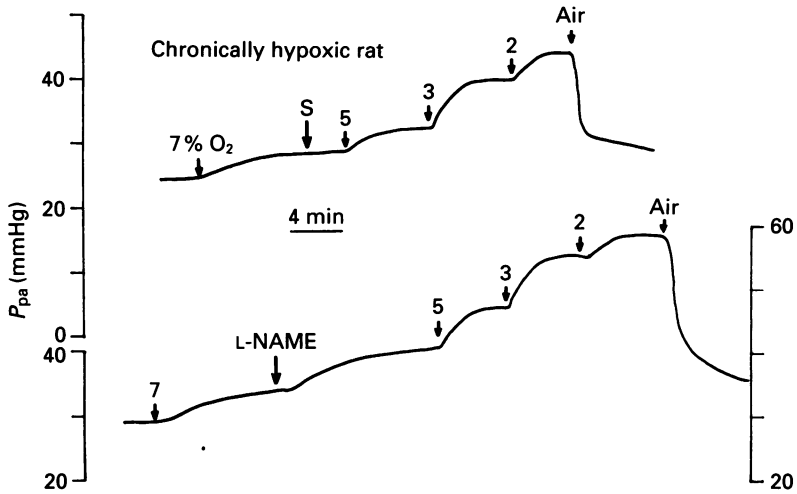

Fig. 8. Trace of pulmonary artery pressure, P_{pa}, showing the effect of L-NAME during hypoxic vasoconstriction in a chronically hypoxic rat. Above, stimulus response to 7, 5, 3, and 2% O_2; 0·9% NaCl was given during ventilation with 7% O_2 (S). Below, a few minutes later, L-NAME given during ventilation with 7% O_2 caused a large further rise in pressure but subsequent rises with more severe hypoxia, show little change compared with the upper trace.

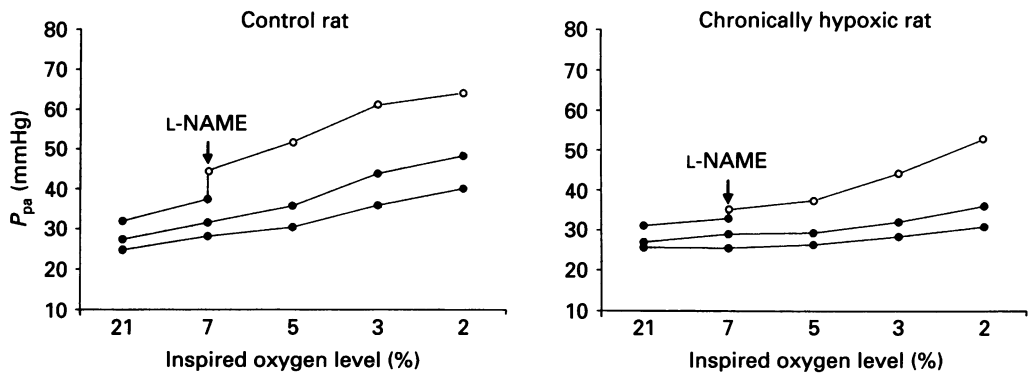

Fig. 9. The two types of change caused by L-NAME in the stimulus–response curve to hypoxia (as in Fig. 8). In each rat three stimulus–response tests to 7, 5, 3 and 2% O_2 were performed, two to show repeatability and a third in which L-NAME was given during 7% O_2 before ((●) and after (○) L-NAME). In both the control rat (above) and the chronically hypoxic rat (below) L-NAME caused a rise in P_{pa}. In the control rat subsequent hypoxic tests did not change the slope of the response to hypoxia. In the chronically hypoxic rat, after L-NAME, there was a steeply rising response to more severe hypoxia.

TABLE 2. L-NAME causes a change in the pressor response to varying degrees of hypoxia; control rats only

		Difference in the pressor response to hypoxia after L-NAME	
% O_2	n	Absolute difference in ΔP_{pa}* (mmHg)	Percentage change in ΔP_{pa}*
7	5	−1·1 ± 2·1	+10·7 ± 35·5 (−59 to +135)
5	7	+7·2 ± 1·8	+106·4 ± 17·1
3	5	+13·6 ± 1·4	+155·4 ± 26
2	8	+18·7 ± 3·9	+124·9 ± 24·4

* Except for 7% O_2, the pressor effect of hypoxia was greater after than before L-NAME. This increase (and the mean change and range for 7% O_2) is recorded in mmHg and as the percentage increase in pre-L-NAME response. All values are means ± S.E.M.

Enhancement by L-NAME of hypoxic vasoconstriction of varying degrees in normal and chronically hypoxic rats

We attempted to measure dose–response relations to a wide range of P_{O_2} values before and after addition of L-NAME. The difficulty lay in establishing a repeatable dose response to hypoxia before NO blockade and before the time lapse had led to a substantial rise in normoxic P_{pa}. We therefore, in some experiments, established a constant response to the least severe hypoxia (7% O_2) and then gave L-NAME during this moderate hypoxia; we also established that 0·9% saline, in a similar volume to L-NAME, had no effect on P_{pa}. Satisfactory results were obtained in seven C and six CH rats, represented by Fig. 8, which is a trace of a CH rat, and Fig. 9 in which we show the two types of response observed. As illustrated by Fig. 9, in both C and CH rats, L-NAME sometimes caused a parallel shift in the dose response to hypoxia and sometimes led to a more steeply rising line.

In further experiments on C rats only, we tested a single hypoxic level before and after L-NAME; the rise in P_{pa} increased with severity of hypoxia (Fig. 8). We found that L-NAME enhanced this pressor response. In Table 2 we give the absolute difference in response caused by L-NAME in mmHg. We have also calculated the *change* in the response as a percentage of the pre-L-NAME value; this varied little for 3, 5 and 2% O_2.

Effect of atrial natriuretic peptide on hypoxic vasoconstriction before and after L-NAME

In six C and six CH rats atrial natriuretic peptide (AMP, 100 ng into the reservoir; approximately 10 ng ml^{-1}) was given during hypoxic vasoconstriction. The dilator effect was calculated as the percentage reduction of the rise in P_{pa} caused by hypoxia. Prior to NO blockade, it was established that the response to ANP was repeatable. Figure 10 summarizes the results. ANP had a greater effect after, than before L-NAME treatment in both groups of rats; C rats, 63·6 ± 6·4 before and 77·3 ± 6·7% after L-NAME ($P < 0·05$); CH rats, 84·2 ± 2·8 before and 119·4 ± 15·4% after L-NAME ($P < 0·01$). Thus, in CH rats after L-NAME, ANP reduced the P_{pa} during hypoxia to a level lower than before the hypoxic test; it abolished the rise in

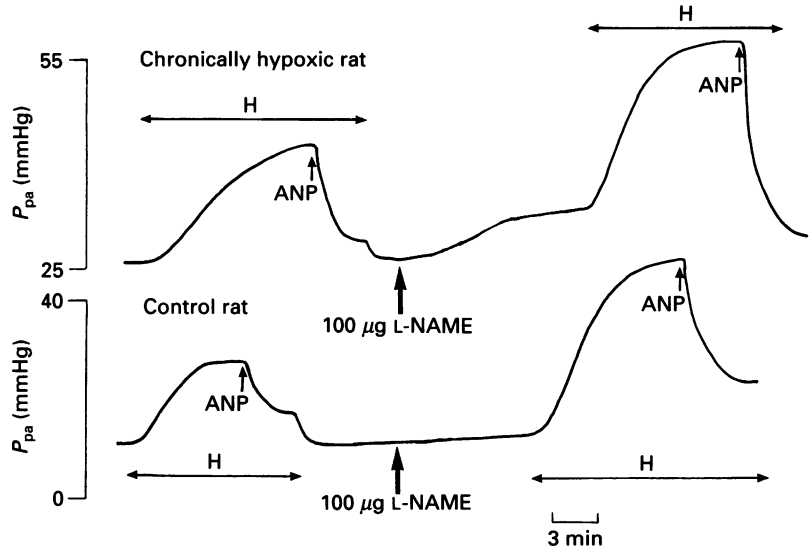

Fig. 10. Schematic record representing the effect of L-NAME on the dilator action of ANP during hypoxia in chronically hypoxic rats (above) and control rats (below). In chronically hypoxic rats, ANP (*ca* 10 ng ml^{-1}) reduced pulmonary artery pressure, P_{pa}, nearly to normoxic levels during hypoxia (H, 2% O_2). L-NAME caused a substantial rise in pressure, followed by an enhanced response to hypoxia. During this hypoxic test, ANP reduced pressure to the level below the pre-hypoxia value. In control rats there was no rise in normoxic pulmonary artery pressure after L-NAME, although the baseline P_{pa} drifted upwards; hypoxic vasoconstriction was enhanced; ANP caused a proportionately smaller dilatation in control than in chronically hypoxic rats.

normoxic pressure caused by L-NAME. The dilator effect of ANP was greater in CH than C rats in percentage terms ($P < 0{\cdot}01$ before L-NAME, $P < 0{\cdot}001$ after L-NAME).

DISCUSSION

During normoxia, blockade of NO synthesis with two analogues of L-arginine caused little or no rise in P_{pa} in control rats but a substantial rise in rats exposed chronically to hypoxia. A tentative conclusion is that NO is continuously released in CH rats and attenuates pressure. Chronically hypoxic rats have a high pulmonary artery pressure, newly muscularized and narrowed arterioles, and higher pulmonary vascular tone than in normoxia (Emery *et al.* 1981). We designed experiments to test which of these features could be responsible for their altered responses to blockade.

Pulmonary arterial pressure was raised passively in control rats by inflating the lung to high pressures. This would raise intravascular pressure in vessels up-stream from the alveolar region. The smallest muscular vessels accompany terminal and respiratory bronchioles and are surrounded by a perivascular space; they are more likely to be expanded by lung inflation than compressed by high alveolar pressure (Howell, Permutt, Proctor & Riley, 1961). These inflations raised pressure above those of chronically hypoxic rats but they did not provoke NO release, since their effects were unaltered after L-NAME. It is thus unlikely that a higher internal pressure in vessels up-stream from the alveolar region is responsible for NO release

in chronically hypoxic rats. As previously observed, chronically hypoxic rats showed a different response to lung inflation, attributed to vascular remodelling (Barer *et al.* 1990*c*).

Pressure was next raised passively in control rats by high rates of flow; this should raise pressure in *all* vessels. Pressure–flow lines were measured. After NO blockade with L-NMMA or L-NAME there was sometimes a slight shift in the line but this displacement was much less than that in chronically hypoxic rats (Fig. 5); at the basal flow rate the changes in pressure were zero or very small (Figs 5 and 6). In the tests with L-NMMA flow raised to 30–40 ml min^{-1}, which raised P_{pa} to approximately 30 mmHg; higher pressures often led to oedema. In further experiments we raised flow up to 70–80 ml min^{-1} which raised pressure to 50–60 mmHg and held it there for *ca* 6 min, despite the danger of oedema. After L-NAME application there was a small rise or no change in pressure at these high flow rates (Fig. 6). Thus, in control rats, raising internal pressure by two methods failed to cause a rise in P_{pa} after L-NAME treatment comparable to the rise found in chronically hypoxic rats. There was no pressure instability at high flow rates.

We next raised P_{pa} actively in control rats by vasoconstriction caused by hypoxia, endothelin 1 and almitrine. In all these circumstances L-NAME led to a further rise in P_{pa}. We concluded that external pressure due to muscle action, rather than passive increases in internal pressure, caused release of NO in normal rats. Figure 7 suggests that a general relationship between the degree of vasoconstriction and the rise in P_{pa} caused by L-NAME may be masked by between-animal variation. Vasoconstriction causes narrowing of vessels, whether anatomically as in CH rats or by constriction as in control rats; both groups release NO. We next considered whether it is the narrowing or the muscle activity that is the most likely cause of NO release.

Moncada and colleagues are of the opinion that high shear stress is a probable stimulus for NO release (Moncada *et al.* 1991). Shear stress is proportional to the pressure drop along a vessel and inversely proportional to the surface area of the vessel lining. When vessels constrict ΔP_{pa} along their length increases and their surface area diminishes due to narrowing; both factors would increase shear stress. High blood flow would only increase the ΔP_{pa} factor. Thus the circumstances in which there is narrowing is vasoconstriction in control rats and the normoxic state in chronically hypoxic rats. In the latter group narrowing is partly due to structural change and partly to basal muscle tone. We suggest that, in our experiments, release of NO was associated in both rat groups with narrowing of small vessels which would alter *both* factors which increase shear stress; internal vascular surface is decreased and the pressure drop along the vessel increased. Shear stress may not, however, be the only stimulus involved. Receptor-operated mechanisms release NO and release has also been detected in isolated vessel preparations and in endothelial tissue culture in the absence of flow (Moncada *et al.* 1991). Other stimuli therefore need consideration. Metabolic activity of pulmonary endothelial cells may be altered in chronic hypoxia; moreover, the environment of endothelial cells in pulmonary arterioles is changed in chronic hypoxia because a new layer of muscle has been laid down externally.

Because both L-arginine analogues gave similar results we are confident that our results were due to blockade of NO synthesis and not to a non-specific action. Since

these analogues compete with L-arginine for NO synthase, their action should be diminished by excess L-arginine. L-arginine partially reversed the effects of L-NMMA but not L-NAME; L-NAME may be more potent than L-NMMA or have irreversible effects on NO synthase but we cannot exclude that it has additional effects. Others have shown that the effects of L-NMMA can be partially reversed by excess L-arginine (Robertson *et al.* 1990*a*).

We measured stimulus–response curves to hypoxia before and after L-NAME. L-NAME caused a greater rise in pressure, the greater the elevation of pressure caused by hypoxia. In percentage terms, the rise caused by L-NAME seemed independent of the hypoxic level. Thus hypoxic vasoconstriction may provoke NO release in proportion to its severity.

Robertson *et al.* (1990*a*) did not find a rise in P_{pa} after addition of L-NMMA in perfused lungs of rats. However, they later showed instability at high flow rates after L-NMMA treatment; pressure rose precipitously (Robertson, Warren & Nye, 1990*b*). This happened at a flow rate of 0·24 ml min^{-1} g^{-1} in their Fig. 1. We did not see instability at similar flow rates in our experiments (Fig. 6). However, very occasionally, we saw instability after L-NAME addition in normal rats during hypoxia at basal flow rates, when the pressure had risen excessively during the course of the experiment.

In contrast to our experiments in isolated lungs, Wiklund, Persson, Gustafsson, Moncada & Hedqvist (1990) found in rabbit lungs, *in vivo*, that L-NAME raised pulmonary artery pressure and pulmonary vascular resistance. They concluded that NO modulates normoxic pulmonary artery pressure and ventilation–perfusion matching. One explanation is that in living rabbits pulmonary arterial P_{O_2} would be lower than in our isolated lung experiments (mixed venous values compared with *ca* 20 kPa; see below). A comparison between *in vivo* and isolated preparations is desirable.

The question has been raised as to whether the low normal pulmonary vascular tone is actively maintained (Weir, 1978). Moncada and colleagues believe that NO may actively and constantly reduce systemic vascular tone (Moncada *et al.* 1991). Is NO therefore responsible for the low pulmonary tone? Our experiments on control rats in normoxia suggest that this is unlikely. However, both arterial and venous vessels are exposed to normoxic P_{O_2} levels in our tests, whereas *in vivo* pulmonary arteries are exposed to venous P_{O_2}. In our experiments in which lungs were ventilated with gas mixtures which would cause 'mixed venous' tensions, NO blockade led to some rise in P_{pa}. However, it is the small pulmonary arteries, probably of diameter 200–300 μm, which constrict during hypoxia. Evidence in man suggests that blood in these vessels is already equilibrated with alveolar air (Jameson, 1964). There is also much evidence in man, in normal conditions, to suggest that pulmonary vessels are in a dilated state. A second point is, were our flow rates comparable to those found *in vivo*? Flow was calculated on the basis of 100 ml min^{-1} kg^{-1}, a higher rate than used by most groups in isolated lungs and one which gives P_{pa} values in the normal range. It is, however, possible that this is a low value for the rat; there are few measurements of cardiac output in this species because of technical difficulties. At twice our flow rate we saw small pressure rises after L-NAME addition but these were much less than those seen in chronically hypoxic rats.

We found no evidence in our rats that chronic exposure to hypoxia impaired nitric oxide synthesis. However, others have found this function attenuated in rats similarly exposed. Adnot, Raffestin, Eddahibi & Chabrier (1991) perfused chronically hypoxic rat lungs with artificial medium. During preconstriction and after pretreatment with meclofenamate to inhibit cyclo-oxygenase synthesis, dilatation in response to acetyl choline and the Ca^{2+} ionophore 23187 was impaired. Although the hypoxic exposure was similar to ours, the isolated lung preparation was profoundly different and might have revealed endothelial changes not detected by us. Perfusion with homologous blood, as in our method, provides a more normal environment for endothelium. Moreover, the preparation used by Adnot *et al.* (1991) was preconstricted with a thromboxane analogue; preconstriction in our tests also provoked NO release.

Dinh-Xuan, Higenbottam, Clelland, Pepke-Zaba, Cremona, Butt, Large, Wells & Wallwork (1991) compared rings of pulmonary artery taken from patients with end-stage chronic obstructive lung disease undergoing heart and lung transplant with those from patients undergoing lobectomy for lung carcinoma. In the latter, considered as 'controls', L-NMMA raised tension further when these rings were precontracted with phenylephrine; in the transplant patients, L-NMMA had no effect. The conclusions were that in the 'controls', NO release was attenuating pressure, whereas in the severe lung disease, this function was impaired. The 'controls' can be compared with our normal rats undergoing vasoconstriction with hypoxia, endothelin, or almitrine. It is also not surprising that the patients with chronic obstructive lung disease had damaged endothelium as the pathology of this condition shows severe intimal changes (Wilkinson, Langhorne, Heath, Barer & Howard, 1988).

In hypertensive pulmonary vascular disease there is anatomical and metabolic evidence for endothelial damage. Dinh-Huan, Higenbottam, Pepke-Zaba, Clelland & Wallwork (1989) found evidence for reduced endothelial-dependent relaxation in isolated rings of pulmonary artery taken from cystic fibrosis patients. Thus synthesis of NO may be impaired and an attenuating influence on pressure lost. In cases where dilator therapy is required it may be necessary to use non-endothelium-dependent drugs. We have shown that the action of ANP does not involve the synthesis of nitric oxide. It caused a reduction in both hypoxic vasoconstriction and in the rise in P_{pa} caused by L-NAME. The dilatory effect of ANP was greater, by the criterion we used, in chronically hypoxic than in normal rats.

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