Pulmonary hypertensive effects of lung inflation in chronic hypoxia: a study in rats

G.R. Barer, P.C. Russell, K. Kapeller Jr


Abstract: Lung inflation was compared in isolated perfused lungs of control (C) and chronically hypoxic (CH) rats; in the latter, there is muscularization and loss of compliance in the pulmonary arterial system.

During ventilation hypoxia, high alveolar pressure (P_{alv}) elevated the pulmonary artery pressure (P_{pa}) less in C than in CH rats; P_{pa} fell during sustained inflation, rose on deflation, and after inflation hypoxic vasoconstriction was attenuated. Opposite changes took place in CH rats; P_{pa} often rose during inflation, fell on deflation, and after inflation hypoxic vasoconstriction was enhanced. Inflation also increased P_{pa} more in CH than C rats during air ventilation. P_{pa}/P_{alv} relations measured during incremental inflation revealed normoxic tone in "extra-alveolar" vessels in both rat groups, which usually increased during hypoxia. In CH, but not C rats, there was also evidence for constriction in "alveolar" vessels during hypoxia. The effects of inflation were not changed by NO synthase blockade in either rat group.

Pulmonary hypertensive effects of inflation in chronically hypoxic rats can be attributed to vascular remodelling.


Evidence suggests that pulmonary vascular resistance is reduced at high lung inflation in the normal individual [1]. The volume of vessels exposed to alveolar pressure is reduced, but that of larger "extra-alveolar" vessels is increased. It is thought that the latter are expanded by radial forces and increasing negative perivascular pressure, whereas the small "alveolar" vessels are compressed or narrowed by increased tension in the alveolar walls; the result is an overall increased lung vascular volume and decreased pulmonary vascular resistance (PVR) [1]. The anatomical identity of the two types of vessels is not defined. However, in normal animals and humans, most of the vessels within the acinus are nonmuscular, whereas in chronic hypoxia, many of them become muscularized [2, 3]. We showed conspicuous changes in reactivity and haemodynamic properties of the pulmonary circulation in chronically hypoxic (CH), compared with normal (C) rats [4–6]. Lung arterioles are also muscularized in hypoxic chronic bronchitis and emphysema, although there are additional features that are found neither in experimental animals nor, with minor exceptions, in high altitude residents [7].

In 1966, Harris et al. [8, 9] showed that patients with chronic bronchitis have an abnormal pressure/flow (P/Q), relation on exercise. Unlike in normal subjects, the "exercise" line is displaced from that measured at rest; in normal subjects, a similar displacement is caused when they breathe against an expiratory resistance. They suggested that, in the patients, airways obstruction might lead to a raise alveolar pressure (P_{alv}) that could lead to a rise in pulmonary artery pressure (P_{pa}). With this work in mind, we looked at the consequences, in CH rats, of raising P_{alv}. We found that during vasoconstriction caused by hypoxia or almitrine, a rise in P_{alv} caused a larger displacement of the P/Q line than in C rats [5, 10]. We attributed this to the remodelling of the chronically hypoxic pulmonary vessels. Our hypothesis was that in chronic hypoxia, lung inflation compresses newly muscularized "alveolar" vessels but fails to expand the stiffer "extra-alveolar" vessels and also that hypoxia constricts more peripheral muscular vessels than in normal rats. However, one feature of this work did not fit the hypothesis quantitatively. We measured the relation between P_{pa} and P_{alv} while we rapidly deflated the lung. In normoxia below an "inflection point" at about P_{alv} 0.27–0.40 kPa (2–3 mmHg) in both C and CH rats, P_{pa} ceased to fall and remained constant or increased. According to the work of Permutt et al. [11], the inflection point reflects the intervention of "extra-alveolar" vessels whose upstream critical closing pressure prevents the transmission of P_{alv} to P_{pa}. Thus, if C rats exhibit hypoxic pulmonary vasoconstriction (HPV) in "extra-alveolar" vessels, the inflection point should rise to a higher P_{alv} during hypoxia: there was a rise, but smaller than expected.

Further work showed that during vasoconstriction caused by either hypoxia or almitrine, high P_{alv} caused greater rises in P_{pa} in CH than C rats and that the pressure often continued to rise [10]. There seemed to be a myogenic response to stretch, the opposite of the normal decrease in PVR with increased lung volume.

In this work, we have compared the relation between lung volume and PVR in C and CH rats and have found...
conspicuous differences. We discuss whether the differences are accounted for by the vascular remodelling of chronic hypoxia and whether they can be attributed to our former or an alternative hypothesis.

**Methods**

Male pathogen-free Wistar rats from the University Field Laboratories were used when ~31 days old. Chronically hypoxic (n=25) rats were kept in a normobaric environmental chamber in 10% O₂ for 3 weeks as previously described [4]. Control (n=20) rats, kept in the same room in air, were tested at the same age as CH rats. We have shown in previous work that CH rats invariably have pulmonary hypertension, right ventricular hypertrophy and muscularization of small arterioles [2–4]. Histology was not performed in this work, but an indication that CH rats had pulmonary hypertension is shown by their higher Ppa at a similar blood flow in perfused lungs (16 C rats (mean±SEM) 2.4±0.12 kPa (17.8±0.88 mmHg)); 21 CH rats 3.4±0.16 kPa (25.7±1.2 mmHg)). Chronically hypoxic rats gained less weight than those kept in air (matched rats, 13 C rats 267±11.8 g, 13 CH rats 239±10.4 g). The number of rats used in each experiment is given in text and tables; some rats were subjected to more than one test.

**Isolated perfused lungs**

After pentobarbitone anaesthesia (60 mg·kg⁻¹ i.p.), the chest was opened and the lungs were perfused with homologous heparinized blood (pH corrected to 7.35–7.45) by means of a Watson-Marlow pump, as previously described [4]. Blood from normal rats was used for both C and CH rat perfusions because the polycythaemic blood of CH rats itself alters lung haemodynamics. The total volume of perfusate was ~10 mL and included a small proportion of haemaccel or dextran. Blood entered the lung through a cannula in the pulmonary artery and left through a second cannula in the left atrium, whence it dripped into a reservoir kept at 38°C and was recirculated. Ppa was measured with a pressure transducer close to the inflow cannula. The lungs were ventilated with air +5% CO₂ (normoxia) or 2% O₂ +5% CO₂ (hypoxia) through a tracheal cannula, by means of a Starling Ideal pump. Blood flow rate was constant at 18–20 mL·min⁻¹; thus changes in Ppa reflected changes in PVR.

**Lung inflations**

For inflation tests, rhythmic ventilation was stopped, and the lungs were inflated/deflated by blowing either air or the hypoxic gas mixture from cylinders over a water trap. This consisted of a T-piece immersed at different depths in a cylinder of water; the inflation or deflation cycle was sustained for 1–2 min. The cycle was repeated several times before ventilation was restored. In a few tests, deflation preceded inflation, and in others, only inflation was tested. In some of these tests (indicated in the text), NO synthase (NOS) was blocked with L-nitro-L-arginine methyl ester (L-NAME, 100 µg into the reservoir, final concentration 10⁻⁵ M). The object of this was to see whether NO might be responsible for any change in Ppa during these manoeuvres; additionally, L-NAME was sometimes given to increase a weak HPV, in order to perform tests during hypoxia. During normoxic ventilation (air+5% CO₂), the lungs were inflated to 2.0 kPa (15 mmHg).

During the plateau phase of hypoxic vasoconstriction, the stroke of the respiratory pump was varied without altering the respiratory frequency.

**Experiment 2: relation between Ppa and Palv during inflation in normoxia and hypoxia vasoconstriction.** The lungs were inflated with either the normoxic or hypoxic gas mixture in 0.3 kPa (2.5 mmHg) steps up to 2.7 kPa (20 mmHg), each pressure was held for 10 s. The relation between Ppa and Palv was plotted. In some of these tests, NO synthase was blocked with L-NAME as in experiment 1.

**Statistics**

Means and standard errors of the mean are quoted. Groups are compared with paired or unpaired Student’s t-tests, as appropriate. Differences were considered significant when the p-value was <0.05.

**Results**

**Experiment 1: comparison of the effect on Ppa of inflation/deflation in C and CH rats during hypoxia and normoxia**

The aim of this experiment was to compare the effect of inflation and deflation in C and CH rats during hypoxia and normoxia (inflation only). Figure 1 shows traces from two C and one CH rat where, during hypoxia, the lung was inflated to a Palv of 2.0 kPa (15 mmHg) and then deflated to a Palv of zero 2–3 times. The long arrows indicate the period of inflation/deflation cycles (during which the chart speed was increased), and the numbers above the trace indicate the Palv. In a C rat (fig. 1a), inflations caused a rise in Ppa of <2.0 kPa (15 mmHg) that then declined, whereas the deflation caused Ppa to fall initially and then climb. Thus, a sustained high inflation pressure led to a fall in PVR and a sustained deflation to a rise in PVR. In a second C rat (fig. 1b), the changes were more extreme. An initial deflation caused a rise in Ppa, a first inflation caused only a fall in Ppa, a second deflation again raised Ppa, whereas the second inflation caused a brief peak and subsequent fall; with each cycle, the Ppa fell progressively so that when ventilation was resumed, Ppa was lower than before the inflation tests and climbed slowly back to near the original value. Thus, hypoxic vasoconstriction was attenuated in some way by the inflation cycles. In the CH rat (fig. 1c) the inflation cycles were characterized by a
larger rise in \( P_{pa} \), which continued to rise during sustained inflation; deflation caused a fall in \( P_{pa} \) and a continued further decline. Note, however, that an initial deflation caused a rise in \( P_{pa} \) as in the C rat (fig. 1b). Thus, with this latter exception, inflation reduced PVR in the C rats but increased it in the CH rat, whereas deflation raised PVR in C rats and decreased it in the CH rat. After the inflation cycles in the CH rat, \( P_{pa} \) was higher than before inflations and declined to near the initial hypoxic value. Inflation enhanced vasoconstriction. (1 mmHg=0.133 kPa.)

**Fig. 1.** – Traces of pulmonary artery pressure (\( P_{pa} \)) changes caused by cyclical inflation (0–15 mmHg alveolar pressure (\( P_{alv} \))) during hypoxic vasoconstriction in two control and one chronically hypoxic (CH) rat (†: start and ∘ finish of hypoxia, 2% \( \text{O}_2 \)). \( P_{alv} \) values are shown (mmHg) above the traces. The chart speed increased during the inflation cycles (↔). a) control rat: at peak constriction, \( P_{alv} \) was cycled 0, 15, 0, 15, 0, 15, and then ventilation was restarted, all during hypoxia; b) control rat: at peak constriction, \( P_{alv} \) was cycled 0–15 mmHg twice. Note that after the inflation cycles, during continued hypoxia, \( P_{pa} \) was reduced and climbed slowly back to near the initial hypoxic level. Inflation attenuated vasoconstriction; c) CH rat: at peak constriction, \( P_{alv} \) was cycled 0–15 mmHg twice. After ventilation, \( P_{pa} \) was higher than before inflations and declined to near the initial hypoxic value. Inflation enhanced vasoconstriction. (1 mmHg=0.133 kPa.)

### Table 1. – Changed effects of inflation/deflation in chronically hypoxic rats

<table>
<thead>
<tr>
<th>Conditions</th>
<th>( \Delta P_{pa} ) mmHg</th>
<th>C rats (n)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH rats</td>
<td></td>
<td></td>
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<tr>
<td>Hypoxia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–15 pre-L-NAME</td>
<td>17.8±1.6 (7)</td>
<td>3.9±0.5 (9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>post-L-NAME</td>
<td>13.3±3.2 (6)</td>
<td>4.3±0.5 (11)</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Sustained inflation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-L-NAME</td>
<td>+9.8±1.4 (8)</td>
<td>-0.4±0.2 (7)</td>
<td>NS</td>
</tr>
<tr>
<td>post-L-NAME</td>
<td>+4.2±2.6 (8)</td>
<td>-1.4±1.2 (7)</td>
<td>NS</td>
</tr>
<tr>
<td>Deflation after inflation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-L-NAME</td>
<td>-2.0±1.3 (6)</td>
<td>+2.1±0.4 (6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>post-L-NAME</td>
<td>-7.1±1.4 (6)</td>
<td>+5.2±1.2 (7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Reventilation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-L-NAME</td>
<td>+0.1±2.4 (5)</td>
<td>+4.7±1.9 (8)</td>
<td>NS</td>
</tr>
<tr>
<td>post-L-NAME</td>
<td>-9.0±3.3 (9)</td>
<td>+4.9±1.9 (10)</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Normoxia</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5–15</td>
<td>10.3±1.5 (7)</td>
<td>7.4±0.5 (7)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Means±SEM are shown. Pulmonary artery pressure (\( P_{pa} \)) and alveolar pressure (\( P_{alv} \)) are in units of mmHg. l-NAME: l-nitroarginine methyl ester; C: control; CH: chronically hypoxic.

in figure 1c (a CH rat) was not reported. Similarly, occasional CH rats showed a decline as in C rats. During hypoxia, after inflation, sustained deflation led to a rise in \( P_{pa} \) in C rats but a fall in CH rats. After reventilation following the inflation cycles, hypoxic vasoconstriction was attenuated in C rats so that \( P_{pa} \) climbed to near the pre-inflation level. However, in CH rats, there was frequently an enhancement of vasoconstriction as shown in figure 1c. \( P_{pa} \) was greater after the inflation cycles and fell slowly to the initial level. However, a proportion of CH rats behaved like C rats; the difference from C rats was only significant in those rats that had received l-NAME, possibly related to the fact that HPV is accentuated by NOS blockade [12]. No other experiments were affected by this blockade. Thus, for example, the decline in \( P_{pa} \) during sustained inflation in C rats cannot be attributed to NO release.

During hypoxia, in six C (three after l-NAME) and seven CH rats (three after l-NAME), instead of sustained cycles, the respiratory pump stroke was increased and decreased to simulate changes in tidal volume (VT). Figure 2c shows a trace from a CH rat. It shows that when the respiratory pump stroke was increased (small ↑ arrows), the \( P_{pa} \) trace became higher and wider, indicating an increase in both mean and respiratory variation, attributable to the increase in tracheal pressure; the reverse took place when the pump stroke was decreased (↓ arrows). In five C rats, the opposite occurred; \( P_{pa} \) decreased with increases in pump stroke and vice versa; in the sixth rat, \( P_{pa} \) fell continually, attributed to a decline in HPV. The pattern seen in the CH rat trace was seen in four CH rats, two more showed this pattern during part of the tests and one behaved like a C rat. Thus, a high proportion of rats exposed to hypoxia showed cyclical changes in PVR in the opposite direction from C rats. Figure 2a and b show these differences; as the pump stroke VT is increased and decreased, the \( P_{pa} \) varies in a cyclical manner, but in the opposite direction in the two rats, and anticlockwise in the CH rat (fig. 2a) clockwise in the C rat (fig. 2b). The l-NAME-treated rat tests were indistinguishable from the untreated rats tests.
The relation between \( P_{pa} \) and \( P_{alv} \) was recorded as \( P_{alv} \) was raised from 0–2.7 kPa (0–20 mmHg) in 0.3 kPa (2.5 mmHg) steps during normoxia and hypoxia. Measurements were made in seven C and seven CH rats (6/7 C and 5/7 CH rats in both normoxia and hypoxia and one CH and two CH rats in hypoxia only); two C and one CH rat had received \( \text{i-NAME} \) before the tests and three C and four CH rats received \( \text{iNAME} \) before a second test. Our aim was to evaluate the contribution of "extra-alveolar" and "alveolar" vessels to basal tone and to hypoxic vasoconstriction, according, to the criteria of the Baltimore group [11]. Figure 3a–c shows tests from three C rats and figure 3d–f three CH rats; in each graph the relation in normoxia is below, and that in hypoxia above. We measured two things: 1) the slope of the linear or near-linear part of the relation at a high \( P_{alv} \) (when close to 1, the slope reflects direct transmission of \( P_{alv} \) to \( P_{pa} \)); and 2) the intercept at low \( P_{alv} \) either as the point at which there was a clear change in slope (as in CH rats in fig. 3) or the point where the linear part intersected with a more gradual curvature at low \( P_{alv} \). This represents the point at which tone in upstream "extra-alveolar" vessels prevents transmission of \( P_{alv} \) to \( P_{pa} \). Figure 3 shows representative traces; in all traces, there is a rise in \( P_{pa} \) in hypoxia; this rise was greater in CH than C rats, as previously observed [4]; figure 3b is an exception, and this rat had received \( \text{i-NAME} \), which enhances HPV in both normal and CH rats [12]. There are clear differences between C and CH rats, but variations in the pattern of response in both groups.

**Inflection points.** All CH rats showed a clear inflection point in normoxia and usually in hypoxia, as the examples in figure 3 show (normoxia \( P_{alv} \) of 0.7±0.08 (5.4±0.6), hypoxia \( P_{alv} \) of 0.9±0.21 kPa (6.7±1.6 mmHg) (mean± SEM)). By contrast, in normoxia, this point was poorly defined in C rats; the relation showed a more gradual curve (estimated value 1.1–0.2 kPa (8.3±1.7 mmHg)). In hypoxia in C rats, there was a curved line in three rats but in three other rats, the pattern seen in figure 3d appeared. At each rise in \( P_{alv} \), \( P_{pa} \) rose and then fell so that at a \( P_{alv} \) of 2.7 kPa (20 mmHg), it was no higher or even lower than at a \( P_{alv} \) of zero; the inflection point could not therefore be calculated. This pattern was not seen in CH rats, except in one where, in a second test after \( \text{iNAME} \), the \( P_{pa} \) had risen >12.0 kPa (90 mmHg). Values for rats treated with \( \text{iNAME} \), both C and CH, fell within the range for untreated rats. Thus, according to our criteria, both C and CH rats showed evidence of "extra-alveolar" vessel tone or constriction in both hypoxia and normoxia.

**Slopes.** Slopes are shown in figure 4. In normoxia, with one exception, the slopes for C rats (0.74±0.18 mmHg·mmHg⁻¹) are less than those for CH rats, which are close to or >1 (0.98±0.15 mmHg·mmHg⁻¹). In hypoxia, C rat slopes, with one exception, were reduced or, as in figure 3a, became effectively zero; the slopes for CH rats...
and b) chronically hypoxic rats during normoxia (N) and hypoxia (H).

Fig. 4. – Slopes of all pulmonary artery pressure (\(P_{pa}\)/alveolar pressure (\(P_{alv}\)) relations at high \(P_{alv}\), as defined in the text. a) control rats and b) chronically hypoxic rats during normoxia (N) and hypoxia (H). ● : untreated rats; ○ : \(N\)-nitro-L-arginine methyl ester treated rats. (1 mmHg·mmHg⁻¹). Except for one C rat, shown in figure 4, \(N\)-NAME treated rats were not different from untreated rats. There was no evidence for "alveolar" vessel constriction in C rats as the slope was <1 in both hypoxia and normoxia. In CH rats, however, the slope was close to 1 both in normoxia and hypoxia. Hypoxia, in most cases, caused a parallel shift in the line at a high \(P_{alv}\), which suggests that constriction in "alveolar" vessels overcame constriction in upstream "ex-tra-alveolar" vessels and \(P_{alv}\) was transmitted to \(P_{pa}\).

Discussion

Despite the complexity and, in some instances, variability of the results, it is clear that inflation had very different effects in C and CH rats, especially during hypoxia. It is probable that the changes in CH rats are related to the remodelling of the vascular bed during hypoxic exposure. The extension of smooth muscle into previously nonmuscular areas within the acinus and the reduced compliance of the arterial system caused by new deposition of connective tissue are likely to be causal.

We found several differences between C and CH rats. In CH rats, the rise in \(P_{pa}\) during inflation was greater than in C rats during hypoxia and often also in normoxia. During hypoxia, sustained inflation frequently led to a continued rise in \(P_{pa}\) in CH rats, whereas in C rats \(P_{pa}\) usually declined. Subsequent to a prolonged inflation, HPV was often enhanced in CH rats but attenuated in C rats. Also, after inflation during hypoxia, deflation was associated with a rise in \(P_{pa}\) in C rats but a fall in \(P_{pa}\) in CH rats. Thus, in CH rats, the normal changes in vascular resistance with lung volume were reversed.

We consider whether these changes in CH rats are related to a peripheral shift in the main site of vasoconstriction to newly muscularized vessels subject to alveolar pressure, to the reduced compliance of their vessels or to the fact that inflation seems to provoke further muscular activity in these rats but to attenuate it in C rats. We use the criteria developed by the Baltimore group to decide whether vasoconstriction took place in "extra-alveolar" or "alveolar" vessels or both [11]. "Extra-alveolar" vessel activity is indicated if there is incomplete transmission of \(P_{alv}\) to \(P_{pa}\) as shown by: 1) a flat or reversed section of the \(P_{pa}/P_{alv}\) curve at low \(P_{alv}\); 2) a curved relation between \(P_{pa}\) and \(P_{alv}\) in the middle \(P_{alv}\) range, attributed to a range of upstream critical closing pressures; or 3) a low slope at high \(P_{alv}\). During vasoconstriction, "extra-alveolar" activity is indicated if the inflection point of the \(P_{pa}/P_{alv}\) line is increased, the lower range of this line becomes more curved and if the slope at high \(P_{alv}\) is reduced. A complicating factor is when inflation attenuates vasoconstriction as it did in C rats. The criteria for alveolar vessel activity are a relation between \(P_{pa}\) and \(P_{alv}\), above the inflection point, close to or greater than 1 and a parallel shift in this relation during vasoconstriction.

Inflation during normoxia

Evidence for tone in "extra-alveolar" vessels in C and CH rats during normoxia. The applied inflation pressure in our tests was due to gas bubbling over a water trap; pressure would, therefore, have been sustained, even if there had been an undetected leak. However, during normoxia, the rise in \(P_{pa}\) during inflation to a \(P_{alv}\) of 2.0 kPa (15 mmHg) was always <2.0 kPa (15 mmHg) in C rats (table 1). This implies that the full \(P_{alv}\) was not transmitted upstream due to some upstream effective or critical closing pressure in "extra-alveolar" vessels. That some basal tone existed in these vessels in C rats is also shown by the flattening or curvature of normoxic \(P_{pa}/P_{alv}\) curves at low \(P_{alv}\) in figure 3. The existence of this tone due to collapsed upstream vessels means that the applied \(P_{alv}\) would cause \(P_{pa}\) to rise only by the difference between upstream critical closing pressure and the applied \(P_{alv}\). The upper part of the \(P_{pa}/P_{alv}\) curve was straight in C rats in normoxia but the slope, with one exception, was <1: this implies that even at a high \(P_{alv}\), it was not fully transmitted upstream. The evidence for tone is surprising because dilator agents have little effect in normoxic C rats [4].

There is similar evidence for tone in "extra-alveolar" vessels in CH rats during normoxia. The rise in \(P_{pa}\) for a \(\Delta P_{alv}\) 2.0 kPa (15 mmHg) was again <2.0 kPa (15 mmHg), although usually greater than in C rats (table 1). All showed some flattening of the \(P_{pa}/P_{alv}\) curve at low \(P_{alv}\) and the rise in \(P_{pa}\), as \(P_{alv}\) was raised further was sometimes gradual, again attributable to a range of upstream critical closing pressures. CH rats show evidence for tone in normoxia as dilator agents have large effects [4]. Also, in a myograph, their isolated arteries of all sizes are in a greater state of tone than those of C rats [13].

Evidence for tone in alveolar vessels in CH rats during normoxia. Above the inflection point in \(P_{pa}/P_{alv}\) curves, which was "lower" but not significantly in CH than in C rats, the relation was straight and the slope near to or >1. Thus, in the high-\(P_{alv}\) range, \(P_{alv}\) was fully transmitted upstream. This does not tell us that there was tone in these vessels because we have no reference line; for this, we would require a line measured during complete relaxation, e.g. after papaverine.
Inflation during hypoxia

Evidence for constriction in "extra-alveolar" and/or "alveolar" vessels during hypoxia in C and CH rats. We think that "extra-alveolar" vessels constricted during hypoxia in both C and CH rats. In C rats, by all three criteria, hypoxic vasoconstriction took place in "extra-alveolar" vessels. During hypoxia in C rats, the increase in Ppa with a 2.0 kPa (15 mmHg) elevation of Palv was very small and very much less than during normoxia (fig. 1, table 1). In the Ppa/Palv curves, the slope was reduced and frequently became zero due to attenuation of hypoxic vasoconstriction (figs. 3a and 4). There was no evidence for constriction in "alveolar" vessels. In CH rats during hypoxia, there was also evidence for constriction in "extra-alveolar" vessels. The flat part of the Ppa/Palv relation became inverted at low Palv, the inflection point was often raised, and the curvature of the Ppa/Palv relation was increased at low Palv. However, there was also evidence for constriction in vessels subject to alveolar pressure. There was a parallel shift in the steep part of the Ppa/Palv relation in many rats, and the slope remained close to 1.

Effects of NOS blockade

After blockade of NOS with L-NAME, we found no change in the responses of C or CH rats to any of the inflation tests. However, in previous work, we found that inflation of the lungs during normoxia caused greater rises in Ppa after NOS blockade in CH, but not, C rats [12]. This result was confirmed in further tests in the current study (not reported); it implies that there is more release of NO for a similar stimulus in CH than C rats.

Effect of inflation on hypoxic vasoconstriction in C and CH rats

As in previous work with our strain of Wistar rat, hypoxia caused a greater constriction in CH than C rats [4]. In other strains, an attenuated response has been found after hypoxic exposure; this may be related to dilator influences as after a few days' recovery in air, an enhanced response appeared, consistent with increased musculature of the vessels [14]. Figures 1a, b and 3a clearly suggest that inflation attenuates hypoxic vasoconstriction in C rats, whereas figure 1c shows that inflation stimulates further vasoconstriction in CH rats. Why should stretch attenuate constriction in C vessels and stimulate constriction in CH rats? We suggest that when the lung is stretched, there is a balance between two effects, a myogenic response and a relaxation of the elastic components of vessels. The balance could be in favour of muscle contraction in CH rats in which all levels of the arterial tree are in an increased state of tone [13] and because the more muscular vessels, stiffened by newly deposited connective tissue, resist elastic relaxation [3, 4]. Our evidence also suggests that inflation causes actual relaxation of smooth muscle during hypoxia in C rats. Alternatively, hypoxia might cause release of a dilator agent in C rats, and this mechanism might be impaired in the damaged vasculature of hypoxic rats. We know that NO is released during hypoxia in normal rats, but in the strain of rats used, we found this mechanism to be enhanced, rather than impaired, after exposure to hypoxia [12]. Whether the observed attenuation/enhancement of hypoxic vasoconstriction during inflation is specific for hypoxia as opposed to other forms of constriction has not been explored.

Comparison of present results with previous work

There are similarities and differences in our current work compared with our earlier work [5]. We have confirmed that inflation during hypoxia leads to a lesser rise in Ppa in CH than C rats, which fits with the smaller shift in the pressure/flow line previously recorded. We attributed this to upstream vasoconstriction in C rats. However, we have now shown that, although hypoxic vasoconstriction does indeed take place in upstream "extra-alveolar" vessels in C rats, inflation attenuates this constriction. Thus, attenuation of constriction could account, at least partly, for the small increments in Ppa during inflation during hypoxia. In C rats, we have shown that hypoxic vasoconstriction occurs in both "alveolar" and "extra-alveolar" vessels. As in C rats, alveolar pressure must first overcome upstream critical closing pressures before Ppa is raised. The greater rises in Ppa in CH than C rats may be compounded by a direct effect of high alveolar pressure on alveolar vessels through stretch or compression and a myogenic response to stretch in unidentified vessels.

The interaction between elastic and muscular activity during inflation in hypoxic conditions is complex, and the exact contribution of each of these factors cannot be assessed. However, it is clear that in control rats, during hypoxic vasoconstriction, inflation leads to a reduction in vascular resistance and deflation to an increase. The reverse usually holds true for chronically hypoxic rats; inflation leads to an increase in resistance, whereas a subsequent deflation reduces resistance. This pattern was usually also observed during rhythmic ventilation with increasing or decreasing pump stroke, which could have implications for hypoxic lung disease. In chronic obstructive pulmonary disease, there is an increased functional residual capacity as well as airway obstruction so that breathing starts from an abnormally high lung volume. We suggest, therefore, that the remodelling of the lung in chronic hypoxic conditions might lead to a change in the effects of deep breathing that could contribute to the known abnormally large rise in pulmonary alveolar pressure on exercise in such individuals. However, in our tests, where the lung was ventilated with positive pressure, the raised alveolar pressure would be during inspiration, whereas in obstructive lung disease alveolar pressure would be low during inspiration but elevated during expiration when the respiratory effort also increases the energy expenditure. Our results support the contentions of Harris et al. [8, 9] made in the 1960s.

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References

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