Hypoxic contraction of small pulmonary arteries from normal and endotoxemic rats: fundamental role of NO

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Hypoxic contraction of small pulmonary arteries from normal and endotoxemic rats: fundamental role of NO. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1207–H1214, 1999.—The present study was aimed at examining the role of nitric oxide (NO) in the hypoxic contraction of isolated small pulmonary arteries (SPA) in the rat. Animals were treated with either saline (sham experiments) or Escherichia coli lipopolysaccharide [LPS, to obtain expression of the isolated small pulmonary arteries (SPA) in the rat. Animals were treated with either saline (sham experiments) or Escherichia coli lipopolysaccharide [LPS, to obtain expression of the inducible NO synthase (iNOS) in the lung] and killed 4 h later. SPA (300- to 600-µm outer diameter) were mounted as rings in organ chambers for the recording of isometric tension precontracted with PGF2α and exposed to either severe hypoxia (bath PO2 8 mmHg) hypoxia. In SPA from sham-treated rats, contractions elicited by severe hypoxia were completely suppressed by either endothelium removal or preincubation with an NOS inhibitor [Nω-nitro-L-arginine methyl ester (L-NAME), 10−3 M]. In SPA from LPS-treated rats, contractions elicited by severe hypoxia occurred irrespective of the presence or absence of endothelium and were largely suppressed by L-NAME. The milder hypoxia elicited no increase in vascular tone. These results indicate an essential role of NO in the hypoxic contractions of precontracted rat SPA. The endothelial independence of HPV in arteries from LPS-treated animals appears related to the extraendothelial expression of iNOS. The severe degree of hypoxia required to elicit any contraction is consistent with a mechanism of reduced NO production caused by a limited availability of O2 as a substrate for NOS.

Methods

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THE IN VITRO CONTRACTILE RESPONSE OF PULMONARY ARTERIES ISOLATED FROM RATS LUNG IN RESPONSE TO PHASE 2 HYPOXIA

The study was approved by the State Committee controlling Animal Experimentation.

In Vivo Part of Experiment

Adult male Wistar rats (250–350 g; IFA-CREDO, Lyon, France) were anesthetized with pentobarbital sodium (50 mg ip) and placed on a heated operation table to maintain esophageal temperature at 37°C. The right femoral vein was dissected and catheterized with PE-50 tubing. Through this femoral catheter, an intravenous bolus of either 10 mg/kg LPS dissolved in 1 ml saline (LPS-treated rats) or the same volume of saline alone (sham-treated rats) was administered in 15 min and followed by a continuous infusion of pentobarbital titrated (10–20 mg·kg−1·h−1) to maintain an appropriate
In Vitro Contractility Studies

The left lung was dissected in EBSS at 4°C with a dissecting microscope and microsurgical instruments. The intrapulmonary bronchial tree was opened and then gently lifted from the underlying arteries. The first-order artery was cut longitudinally to allow threading of a thin wire (80 µm) into the lumen of second-order branches for in situ endothelial removal when required. The third and fourth branches (counting from the hilus) were systematically used. The rings were transferred into organ chambers heated at 37°C and mounted on two vertical stainless steel needles (diameter 80 µm). One of the needles was directly attached to the chamber floor and the other to a strain gauge (UL20-GR, Minebea) for the measurement of isometric force.

The 2-ml chambers were continuously perfused with EBSS containing L-arginine (10^{-4} M) and genticamin (2.5 mg/ml). EBSS was gassed with the required concentration of O_2 plus 6% CO_2 in N_2, to obtain a pH between 7.36 and 7.40. Gassed EBSS was supplied from a pair of heated 50-ml reservoirs, one bubbled with 21% O_2 and the other with a hypoxic mixture (either 2.5% or 0%). The liquid was transferred in and out of the chambers at a constant rate of 4 ml/min by means of two peristaltic pumps (IPN4, Ismatec, Zürich, Switzerland). Outflow occurred through an opening located above the chamber floor at a maximal rate set higher than the inflow rate so that the volume of fluid within the chamber remained constant (2 ml). Depending on the protocol stage (see below), the outflow was either discarded (open-circuit configuration) or recyled to the reservoirs (closed circuit). The chamber lid, made of Plexiglas, had an opening for the transducer arm. To minimize contamination by room air, we continuously flushed the gas phase within the chamber with 60 ml/min of a mixture identical in composition to that used for the gassing of EBSS. All inflow tubings were stainless steel except the plastic tubes inside the peristaltic pump. At any time, the chamber PO_2 could be measured by directing part of the outflow (1.6 ml/min) through stainless steel tubing to an oxygen electrode (OX1100, Schott Geräte, Hofheim, Germany) placed in-line within the circuit, 5 cm downstream from the chamber. Immediately after each PO_2 reading, calibration was checked by driving fluid previously gassed for at least 30 min with either 0% or 21% O_2 through a path made entirely of stainless steel tubing, into the electrode at the same flow rate used for measurement. Chamber hypoxia was achieved in the closed-circuit configuration by rapidly switching perfusion from the normoxic to the hypoxic reservoir. Chamber PO_2 stabilized in 10 min, with 90% of the change occurring in the first 3 min.

In many similar studies, the chamber PO_2 has been measured with a blood gas analyzer (BGA) rather than an in-line electrode (1, 14, 34). We preferred the latter method because, considering the limited solubility of O_2 in water, samples of hypoxic electrolyte solutions could easily be contaminated by the atmospheric PO_2 when introduced into a BGA. Indeed, we compared the values of bath PO_2 simultaneously obtained from the in-line electrode and from a BGA (AVL 945) installed in the same room, thus allowing transfer of samples within a few seconds using a glass capillary tube. The PO_2 measured with both methods (expressed in mmHg as mean ± SE of 3 determinations) agreed closely in normoxic (EBSS gassed with 21% O_2: BGA 142 ± 0.6, in-line electrode 140 ± 0.3) but not in hypoxic (2.5% O_2: BGA 48 ± 0.8, in-line electrode 22 ± 0.0; 0% O_2: BGA 35 ± 0.8, in-line electrode 7 ± 0.6) conditions. Even when measured with the in-line electrode, the chamber PO_2 in hypoxia was somewhat higher than predicted on the basis of O_2 content in the gas used for equilibration, likely reflecting contamination by atmospheric O_2 downstream from the reservoirs.

After being mounted into the organ chamber, the arteries were left to rest for 30 min and then stretched to a passive tension equivalent to a transmural pressure of 15 mmHg for vessels of that size, according to a previously described procedure (13). The transmural pressure was chosen to approximate the normal value of pulmonary artery pressure in vivo. Preliminary experiments also showed that this degree of preload was optimal, i.e., it led to maximal force development after exposure to 80 mM KCl. Thirty minutes after stretching, the following sequence of steps was initiated. 1) The maximal tension developed during a 10-min exposure to 80 mM KCl (T_max) was determined. 2) A partial cumulative concentration-response curve to PGF_2α, was constructed until the tone exceeded 20% of T_max, ACh (10^{-5} M) was then added to the bath and the induced relaxation was taken as an index of endothelial integrity. 3) In experiments requiring the inhibition of NOS, the liquid in the whole circuit was replaced by a solution of the same composition with the addition of L-NAME (10^{-3} M, pH adjusted to 7.36–7.40). 4) Whether or not L-NAME had been added at step 3, a second partial concentration-response curve was obtained, from which the PGF_2α concentration able to induce an active tension equal to 20% of T_max (EC_{50}) was determined. 5) These arteries required a certain amount of preinduced tone to respond to hypoxia (13, 14); therefore, a concentration of PGF_2α equal to the EC_{50} was added to the liquid in the circuit and then slightly adjusted if necessary until a stable precontraction between 20 and 35% of T_max was obtained. 6) The vessels were exposed to hypoxia for 35 min and then reoxygenated. At the end of each experiment, the arteries were systematically fixed in 4% Formalin. Subsequent standard histological examination was performed in some intact and endothelium-denuded vessels.

All steps before step 6 were carried out in normoxia. The partial cumulative dose-response curves (steps 2 and 4) were obtained by stopping flow through the circuit and then sequentially adding increasing amounts of PGF_2α dissolved in EBSS directly into the 2-ml chamber through a small hole in the lid to achieve the following concentrations: 10^{-6}, 3 × 10^{-6}, 10^{-5}, 3 × 10^{-5}, and 10^{-4} M. Thorough mixing was ensured by continuous stirring for 3 min before tension was read. In step 2, the procedure was stopped as soon as tension exceeded 20% of T_max. In step 4, only the three lowest concentrations of PGF_2α were systematically tested; the higher ones were given only if required to obtain a tension >20% of T_max. We chose not to complete the dose-response curves because tension frequently did not plateau at 10^{-4} M PGF_2α, and because the use of higher concentrations would have been extremely expensive.
In preliminary experiments, we determined that the pattern of hypoxic contraction was the same with either KCl or PGF\textsubscript{2\alpha}, as the precontracting agent (data not shown). PGF\textsubscript{2\alpha} was chosen because of the stable tension achieved with this agent in step 5.

Two rings per rat were studied, one with and the other without L-NAME. Both rings from the same animal were either endothelium intact or endothelium denuded.

Two series of experiments were successively performed with identical protocols, except for step 6. In series 1, two levels of hypoxia (2.5% and 0% \textsubscript{O}_2) were applied for 35 min each, with 15 min of reoxygenation in between. The milder hypoxia was applied first. In series 2, a single 35-min severe hypoxia (0% \textsubscript{O}_2) was applied. Series 2 was carried out to exclude the possible influence of ischemia-reperfusion events on the contractile response to 0% \textsubscript{O}_2.

### RESULTS

#### iNOS Activity

Rats were treated and killed as described in In Vivo Part of Experiment. All second-order arteries from the left lung were dissected, immediately frozen in liquid nitrogen, and stored at −80°C. Subsequently, iNOS activity was determined in tissue homogenates from the calcium-independent conversion of L-[3H]arginine to L-[3H]citrulline using a previously described micromethod (24). Only endothelium-intact arteries were studied.

#### Solutions and Chemicals

All reagents were from Sigma (Buchs, Switzerland) with the exception of PGF\textsubscript{2\alpha} (Cayman Chemical, Ann Arbor, MI). PGF\textsubscript{2\alpha} (10–3 M in EBSS) and ACh (5.5 × 10–3 M in saline) stock solutions were prepared, aliquoted, and stored at −20°C. The working solutions were prepared each day from either dry powder or frozen aliquots, as appropriate. EBSS had the following composition (in mM): 116.3 NaCl, 5.3 KCl, 26.1 NaHCO\textsubscript{3}, 1.8 CaCl\textsubscript{2}, 1.0 NaH\textsubscript{2}PO\textsubscript{4}, 0.8 MgSO\textsubscript{4}, 5.5 d-glucose, and 0.03 phenol red (sodium salt; as pH indicator). The 80 mM KCl solution used for the determination of T\textsubscript{max} was identical to EBSS except for the isosmolar substitution of KCl for NaCl. L-Arginine (10–4 M) and gentamicin (2.5 mg/ml) were systematically added to both EBSS and 80 mM KCl.

#### Experimental Design and Data Analysis

All designs were full factorial with randomization. As explained, different arteries from the same animal were tested in the presence or absence of L-NAME, and endothelium-intact and endothelium-denuded vessels always came from different animals. Results were analyzed accordingly, using the appropriate analysis of variance model. When the F-value for an effect was globally significant, planned pairwise comparisons were made with modified t-tests (i.e., Fisher’s protected least significant difference). The α-level of all statistical tests was set at 0.05. Computations were performed with the JMP software (SAS Institute, Cary, NC). All data are given as means ± SE unless stated otherwise. Sample size is systematically indicated.

### RESULTS

#### In Vitro Contractility of SPA

Table 1 shows mean T\textsubscript{max} and responses to ACh obtained in the two series of experiments. T\textsubscript{max} was lower in endothelium-denuded compared with endothelium-intact vessels, although this effect only reached statistical significance in one subgroup of series 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>T\textsubscript{max}, mm</th>
<th>ACh Relaxation, % of preinduced tension</th>
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<tbody>
<tr>
<td>Series 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E+</td>
<td>1.17 ± 0.08</td>
<td>71.8 ± 3.6</td>
</tr>
<tr>
<td>E−</td>
<td>0.61 ± 0.04</td>
<td>8.0 ± 2.9†</td>
</tr>
<tr>
<td>LPS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E+</td>
<td>0.77 ± 0.12*</td>
<td>38.2 ± 2.2†</td>
</tr>
<tr>
<td>E−</td>
<td>0.63 ± 0.06</td>
<td>−4.0 ± 2.9‡</td>
</tr>
<tr>
<td>Series 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E+</td>
<td>0.88 ± 0.04</td>
<td>73.2 ± 1.5</td>
</tr>
<tr>
<td>E−</td>
<td>0.65 ± 0.10</td>
<td>4.6 ± 3.1‡</td>
</tr>
<tr>
<td>LPS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E+</td>
<td>0.64 ± 0.08*</td>
<td>38.8 ± 3.4†</td>
</tr>
<tr>
<td>E−</td>
<td>0.47 ± 0.05*</td>
<td>−8.7 ± 2.1†</td>
</tr>
</tbody>
</table>

Data are means ± SE of 9–10 arteries taken from 4–5 rats. Maximal active tension of small pulmonary arteries in response to 80 mM KCl (T\textsubscript{max}) and relaxation response to 10−5 M acetylcholine (ACh) administered after submaximal precontraction with prostaglandin F\textsubscript{2α}. Arteries of series 1 were subsequently exposed to 2.5% \textsubscript{O}_2 followed by 0% \textsubscript{O}_2; arteries of series 2 were subsequently exposed to 0% \textsubscript{O}_2 only. Sham and LPS, animals treated with saline or lipopolysaccharide (LPS). E+ and E−, endothelium intact or removed. In endothelium-denuded vessels from rats treated with LPS, ACh induced a small increase in tone (negative values for ACh relaxation). *P < 0.05, †P < 0.01 compared with same conditions in absence of LPS; ‡P < 0.01 compared with same conditions in presence of endothelium.

Standard histological examination of endothelium-denuded rings (not shown) revealed no visible damage to the smooth muscle layer. We did observe a small number of breaks in the lamina elastica interna, which nevertheless remained clearly visible and sharply delineated around most of the intimal circumference. Mean T\textsubscript{max} was generally somewhat lower in the presence than in the absence of LPS treatment.

In endothelium-intact vessels from sham-treated rats, 10−5 M ACh caused an immediate and intense relaxation. This response was blunted, although still clearly obtained, in vessels from LPS animals. As expected, endothelium removal essentially abolished any vasomotor effect of ACh.

Figure 1 shows the influence of the various conditions, in normoxia, on sensitivity of the rings to the vasocostrictor effect of PGF\textsubscript{2α}. Pooled data are presented for brevity, because behavior in the two series of experiments was homogeneous. In the absence of prior treatment with LPS, EC\textsubscript{20} was drastically reduced (almost 10-fold) by endothelium removal. In endothelium-intact rings, a reduction of the same magnitude was achieved with the NOS inhibitor L-NAME. By contrast, L-NAME did not change the EC\textsubscript{20} of endothelium-denuded rings. Treatment of the animals with LPS was associated with a large increase in EC\textsubscript{20} in both the presence and the absence of endothelium. This effect of LPS was largely reversed by L-NAME, although not completely in endothelium-intact rings.
Fig. 1. Vasoconstrictor effect of PGF$_{2\alpha}$ on small pulmonary arteries. EC$_{50}$, concentration of PGF$_{2\alpha}$ required to elicit 50% of maximal active tension generated in response to 80 mM KCl (T$_{max}$): sham and LPS animals treated with saline or LPS, respectively, in vivo: E + and E −, endothelium intact or endothelium removed in vitro; L-NAME − and L-NAME +, absence or presence of N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME; 10$^{-4}$ M) in vitro. In all conditions, organ bath contained L-arginine (10$^{-4}$ M). Data are pooled from 2 series of experiments. Each column and error bar is mean ± SE. **P < 0.01 compared with same conditions in absence of treatment with LPS; §§P < 0.01 compared with same conditions in presence of endothelium; †P < 0.01 compared with same conditions in absence of L-NAME.

As usual in this preparation (14, 29, 35), hypoxic contractions could not be obtained without the prior induction of submaximal active tension with a vasoconstrictor agonist, and PGF$_{2\alpha}$ was used for that purpose (precontraction, step 5 of the protocol). By design, the mean level of precontraction was relatively uniform between experimental groups (lowest and highest values, expressed as % of T$_{max}$: 22 ± 1 and 30 ± 2). Thus the concentrations of PGF$_{2\alpha}$ used for precontraction (i.e., present in the bath during hypoxia) varied between conditions, according to the pattern predicted by Fig. 1. Precontraction tone was absolutely stable during the 10 min preceding hypoxia (not shown).

The effects of two levels of hypoxia on vessel tone in the various conditions are displayed in Figs. 2 and 3. Major changes in tone only occurred on equilibration of the organ bath with 0% O$_2$ (PO$_2$: 8 ± 3 mmHg (mean ± SD)) but not with 2.5% O$_2$ (21 ± 3 mmHg).

Severe hypoxia (0% O$_2$). The effects of 0% O$_2$ were essentially the same in the two series of experiments. Only results from series 2 are presented here (Fig. 2). In the absence of treatment with LPS, severe hypoxia induced a typical pattern consisting of a transient contraction (phase 1) followed by transient partial relaxation and then by a sustained progressive contraction starting 15–20 min after the onset of hypoxia (phase 2). Phase 2 contraction was rapidly reversed on reoxygenation (not shown). This time course was profoundly altered by endothelium removal, exposure to L-NAME, or both, with no difference noted in the effects of these three interventions: phase 1 and phase 2 contractions were abolished and replaced by progressive relaxation. There was no statistically significant effect of treatment with LPS on the hypoxic contraction induced by 0% O$_2$ in endothelium-intact rings not exposed to L-NAME. However, in sharp contrast with findings in the absence of LPS, the hypoxic contraction was not abolished or even significantly modified by endothelium removal. The hypoxic contraction of vessels from endotoxemic animals was markedly suppressed by L-NAME. This suppression was total in endothelium-denuded rings, whereas endothelium-intact rings exposed to L-NAME retained at all time points a hypoxic tone slightly higher in the presence compared with the absence of treatment with LPS.

Milder hypoxia (2.5% O$_2$). As stated, there was essentially no contraction with this level of hypoxia (Fig. 3). There were, however, some statistically significant differences between conditions, all of which were in the same direction observed with severe hypoxia, although of much smaller amplitude.

iNOS Activity

This activity, as measured from the calcium-independent conversion of labeled L-arginine into L-citrulline in homogenates of endothelium-intact SPA, was massively enhanced by in vivo treatment with LPS (Fig. 4).

Fig. 2. Effects of severe hypoxia (0% O$_2$) on active tension of small pulmonary arteries in saline (sham; A-) and lipopolysaccharide (LPS; B-) treated rats (experiments of series 2). Before hypoxia, arteries were precontracted with PGF$_{2\alpha}$, titrated to achieve, in normoxia, a tone between 20 and 35% of T$_{max}$. In all conditions, organ bath contained L-arginine (10$^{-4}$ M). Data are from experiments in series 2 only (severe hypoxia in series 1 gave similar results). Each point is mean ± SE of 4–5 arteries (1 artery/rat). *P < 0.05 compared with same conditions in absence of treatment with LPS; §P < 0.05 compared with same conditions in presence of endothelium; †P < 0.05 compared with same conditions in absence of L-NAME.

Fig. 3. Effects of milder hypoxia (2.5% O$_2$) on active tension of small pulmonary arteries in sham (A-) and LPS (B-) treated rats (experiments of series 1). Each point is mean ± SE of 4–5 arteries (1 artery/rat). P values as in Fig. 2.
As mentioned (see METHODS), our standard procedure was to use PGF2\textsubscript{a} to elicit these contractions. In endothelium-intact vessels, the vigorous relaxation elicited by ACh argues against this possibility. Furthermore, additional experiments (not shown) indicated that rings sampled more distally than in the principal experiments had a substantially higher T\textsubscript{max}. With the endothelium intact, L-NAME drastically reduced but did not completely abolish the difference in T\textsubscript{max} induced by LPS treatment in both endothelium-intact and endothelium-denuded rings. The reversal by L-NAME of LPS-induced hypocontractility of rings from LPS-treated rats may have been augmented by the presence of 100 µM L-arginine in the bath, because production of NO in tissues expressing iNOS is dependent on an exogenous supply of this substrate (3, 26).

**DISCUSSION**

The principal finding of this study was the complete suppression by NOS inhibition of both phase 1 and phase 2 hypoxic contraction in precontracted SPA in the rat. Furthermore, both phases required an intact endothelium in the absence but not in the presence of prior in vivo treatment with LPS. Finally, severe hypoxia (i.e., a bath PO\textsubscript{2} well below 20 mmHg) was required to elicit these contractions.

Contractile Behavior in Normoxia

Sham experiments. Under all conditions, T\textsubscript{max} was lower than usually reported in pulmonary arteries of this size (13, 14, 29, 35; Table 1), raising the concern of possible tissue damage inflicted in the course of our study. In endothelium-intact vessels, the vigorous relaxation elicited by ACh argues against this possibility. Furthermore, additional experiments (not shown) indicated that rings sampled more distally than in the principal experiments had a substantially higher T\textsubscript{max}. As mentioned (see METHODS), our standard procedure was to excise rings from second-order branches in close proximity to the junction with the first-order artery. In this location, smooth muscle cells might either be less abundant or have a noncircular orientation in comparison with a more distal site. Some damage to smooth muscle may have been present in endothelium-denuded arteries, which had a lower T\textsubscript{max} than their endothelium-intact counterparts, especially in the experiments of series 1 (Table 1); in series 2, this effect of endothelium removal was smaller and not statistically significant. Rings in which the endothelium had been rubbed had only very limited histological evidence of damage and did disclose the expected increase in sensitivity to a vasoconstrictor agonist (i.e., a lower EC\textsubscript{50} for PGF\textsubscript{2a}; Fig. 1).

In endothelium-denuded arteries, L-NAME did not modify the EC\textsubscript{50} for PGF\textsubscript{2a}. In endothelium-intact vessels, in contrast, inhibition of NOS increased the sensitivity to PGF\textsubscript{2a} severalfold, indeed to exactly the same extent as did endothelium removal (Fig. 1). Taken together, these observations indicate that, in agreement with the scant data available in rat SPA (14, 29), the L-arginine-NO pathway plays an essential role in the modulation of vascular tone by the endothelium under the conditions of the present study.

Effects of treatment with LPS. In many species, in vivo treatment with LPS has a depressor effect on vascular contractility. In rat isolated aortas (3, 6, 10, 31), other systemic vessels (25), or large extrahilar pulmonary arteries (6, 34), this defect has been related to hyperactivation of the L-arginine-NO pathway caused by the stimulated expression of iNOS in the vascular wall. Our results provide two arguments for the same scenario in SPA. Four hours after LPS treatment, iNOS was massively expressed in these vessels (Fig. 4). Furthermore, the marked decrease in sensitivity to the vasoconstrictor action of PGF\textsubscript{2a} induced by LPS treatment in both endothelium-intact and endothelium-denuded rings was essentially reversed by L-NAME (Fig. 1). The hypococontractility of rings from LPS-treated rats may have been augmented by the presence of 100 µM L-arginine in the bath, because production of NO in tissues expressing iNOS is dependent on an exogenous supply of this substrate (3, 26). The precise sites of iNOS induction in the vascular wall cannot be directly ascertained from our data because, for technical reasons, iNOS activity could only be determined in intact arteries. Nevertheless, a strong indication for an extraendothelial site is given by the differential effect of L-NAME on the EC\textsubscript{50} to PGF\textsubscript{2a} in endothelium-denuded rings from LPS- versus sham-treated rats (Fig. 1). Concomitant enzyme induction in the endothelium could also have occurred (11).

The reversal by L-NAME of LPS-induced hypocontractility was complete in endothelium-denuded vessels. With the endothelium intact, L-NAME drastically reduced but did not completely abolish the difference in sensitivity to PGF\textsubscript{2a} between rings from LPS- and sham-treated rats. Assuming that LPS stimulated the expression of iNOS in the endothelium, this residual difference could be related to the lower potency of L-NAME as an inhibitor of iNOS, as opposed to constitutive endothelial NOS (ecNOS) (28). Alternatively, LPS could have stimulated the endothelial production of a vasodilator unrelated to NO. Whatever the explanation, the magnitude of LPS effects not inhibited by L-NAME was relatively small. The blunting by LPS of T\textsubscript{max} and of endothelium-dependent vasodilation to ACh is in accordance with a large body of experimental work in different vessels and different species (18, 25, 31, 32).

Hypoxic Contractile Responses

Level of hypoxia required to elicit contraction in vitro. A first feature of all hypoxic contractions observed in the present study was the very low PO\textsubscript{2} required to elicit these responses. Indeed, the vigorous contractions observed with severe hypoxia (bath PO\textsubscript{2} 8 ± 3 mmHg; Fig. 2) were essentially absent with a slightly higher PO\textsubscript{2} (21 ± 3 mmHg; Fig. 3). The ability of severe hypoxia to
elicited contraction of isolated pulmonary arteries is consistent with a substantial amount of experimental work in the rat (13, 17, 23, 29) and other species (8, 12). On the other hand, the lack of response to a slightly milder hypoxia may seem to contradict several reports of contractions obtained in isolated rat pulmonary arteries submitted to a bath PO₂ of 23 (34), 33 (1, 14), 44 (23), or even 57 (33) mmHg.

There are two nonmutually exclusive explanations for this apparent discrepancy. First, the bath PO₂ may have been overestimated in some studies (1, 14, 34) because of the use of a BGA rather than an in-line PO₂ electrode, as explained in METHODS. This potential error did not occur in the study by Rodman and associates (23). However, as these authors note, extrapolation from bath to cellular PO₂ may be difficult because of the problems of unstirred layers and diffusion barriers. Such problems may have been minimized in the present work because of the continuous perfusion of organ chambers during hypoxia and the small size of the arteries examined. Viewed against this background, our data strongly support the idea that a severe level of hypoxia, indeed, close to anoxia, is required to trigger a further increase in the tone of precontracted rat SPA.

Sham experiments. In our experiments, the contraction elicited by severe hypoxia in intact preconstricted vessels followed the typical biphasic pattern reported by other studies of either large (1, 14) or small (13, 14, 29) pulmonary arteries in the rat (Fig. 2). Both the early transient phase 1 and the later sustained phase 2 were completely suppressed by removal of the endothelium. In the case of phase 2, this is consistent with most observations made by others (13, 14, 33, 35). On the other hand, phase 1 was either partially (13, 14) or totally (35) endothelium independent in studies of small arteries, including one from our own laboratory (13), at some variance with our present findings. The reasons for this discrepancy are not clear. It could be caused by the possible damage inflicted on smooth muscle during endothelium removal, as discussed in Contractile Behavior in Normoxia. The suppression of phase 1 by L-NAME in endothelium-intact vessels (discussed below) argues against this possibility. Alternatively, unrecognized variables could be involved, such as the exact kinetics of PO₂ change or the genetic background of the animal.

Several studies in large rat pulmonary arteries found that NOS inhibitors or scavengers of NO abolish (5, 22, 33) or diminish (17) phase 1 and suppress phase 2 (33). Analog information in rat SPA is less abundant. Teng and Barer (29) reported a considerable blunting of phase 1 contraction when such arteries were preincubated with L-NAME (100 µM) but provided no information on phase 2. The present experiments support and complement this study: not only phase 1 but also phase 2 was totally abolished by L-NAME (1 mM) (Fig. 2). This cumulated evidence obtained with pharmacological probes implies that the L-arginine-NO pathway plays a fundamental role at all phases of hypoxic contractions in both large and small pulmonary arteries from nonendotoxemic rats.

At variance with this conclusion, Leach and co-workers (14) found that neither phase 1 nor phase 2 was affected by the NOS inhibitor Nω-nitro-L-arginine (L-NNA), whether in large or small rat pulmonary arteries. These authors used a relatively low concentration of L-NNA (10 µM), which efficiently blocked relaxation to Ach but failed to significantly augment the contractile response to a vasoconstrictor agonist, in contradistinction to effects noted by others (3, 6, 26, 29, 33, 34) and ourselves (Fig. 1) with higher concentrations (100 µM to 1 mM) of various L-arginine analogs including L-NNA (5, 31). Dose-response studies in rat aortas indicate that higher concentrations of NOS inhibitors may be required for the augmentation of agonist-induced contraction, as opposed to the inhibition of endothelium-dependent relaxation (19), raising the possibility that basal and agonist-stimulated endothelial production of NO may be differentially affected by NOS inhibitors (20). In short, results by Leach and co-workers (Fig. 9 in Ref. 14) would be reconciled with ours (Fig. 2) if it is assumed that in their study NOS inhibition was insufficient to suppress the basal release of NO.

Effects of treatment with LPS. The effects of in vivo endotoxemia on the hypoxic contractile behavior of isolated pulmonary vessels have been explored for the first time in the present study. We found that precontracted SPA from LPS-treated rats retained the ability to increase tone on severe hypoxia (Fig. 2). In contrast with observations in arteries from sham-treated rats, this hypoxic contraction was not altered by endothelium removal. There was a remarkable parallel between the effects of NOS inhibition on the sensitivity to PGF₂α in normoxia (Fig. 1) and on the time course of vascular tone during severe hypoxia (Fig. 2): whenever L-NAME decreased the EC₂₀ (i.e., in all conditions except endothelium-denuded arteries of sham-treated rats), it also suppressed or largely diminished the subsequent contraction in response to severe hypoxia. These data imply a major role of the L-arginine-NO pathway in all hypoxic contractions observed in the present study. The endothelium independence of hypoxic contractions in arteries from LPS-treated rats simply reflects the activation of this pathway at an extraendothelial site, presumably because of the expression of iNOS, as discussed in Contractile Behavior in Normoxia. This result is consistent with the work of Zelenkov and co-workers (34), who found in the rat that endothelium-denuded large pulmonary arteries did not contract on hypoxia unless incubated with LPS in vitro. We extended these findings in two ways: we demonstrated such effects in more peripheral pulmonary arteries differently exposed to LPS (i.e., in vivo), and we showed that, at least in our conditions, the PO₂ required to trigger hypoxic contraction was not modified by treatment with LPS (Figs. 2 and 3).

Further Comments

The nature of the link between the L-arginine-NO pathway and the hypoxic contractions observed in precontracted pulmonary arteries is uncertain. In
theory, hypoxia could either inhibit NOS or act distally to the generation of NO. Recently, the effects of O$_2$ concentration on NOS activity have been investigated, using purified bovine aortic eNOS and iNOS from cultured RAW 264.7 macrophages; for these two isoforms of NOS, the Michaelis constant values for O$_2$ were 7.7 and 6.3 µM, respectively, equivalent under normal atmospheric pressure to PO$_2$ of 6 and 5 mmHg (21). If rat isoforms of NOS behave similarly, severe hypoxia under the conditions of our experiments (i.e., a bath PO$_2$ of 8 mmHg, necessarily associated with a lower intracellular PO$_2$ because of diffusion barriers) would obviously inhibit NO production by the mere limitation of the O$_2$ substrate, whereas this effect would be less likely with milder hypoxia (bath PO$_2$ 21 mmHg). Thus, although inhibition of NOS by severe hypoxia cannot be ascertained from our data, it would be consistent with the differential effects of the two levels of hypoxia tested in the present study. Further studies would be required to dissect out the interactions of hypoxia with the L-arginine-NO pathway in vascular tissue.

In conclusion, our data highlight several important differences between hypoxic contractions exhibited in vitro by precontracted SPA in the rat and the usual characteristics of HPV in the intact lung. A much lower PO$_2$ is required to elicit vasoconstriction in vitro than in perfused rat lungs (16) or in vivo (2). Furthermore, although pharmacological NOS inhibitors abolish or reduce HPV in isolated rat arteries, they enhance it in the intact lung (7, 15). Finally, in contrast with several observations made in the intact pulmonary circulation (4, 30), in vivo endotoxemia does not suppress hypoxic contraction in isolated endothelium-intact arteries. These differences must be borne in mind when using isolated pulmonary arteries in the rat as a tool to investigate the mechanisms of HPV.

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REFERENCES


