Hypoxic Pulmonary Vasoconstriction Is Not Endothelium Dependent (43506)

C. MARSHALL[1] AND B. E. MARSHALL
Center for Anesthesia Research, University of Pennsylvania, Philadelphia, Pennsylvania 19104-4283

Abstract. **Feline intrapulmonary arteries (mean diameter, 0.9 mm) were equilibrated in Earle's solution at constant tension in a chamber bubbled with an hyperoxic gas mixture (30% oxygen, 5% carbon dioxide, balance nitrogen). The endothelium was removed from half the vessels by gentle rubbing. The isometric response to the addition of acetylcholine ($1*10^{-6}$ *M*) was dilator in the vessels with endothelium and constrictor in those without endothelium. Intermittent exposure to a hypoxic gas mixture (0% oxygen, 5% carbon dioxide, balance nitrogen) for 20 min with five repetitions demonstrated sustained constrictor responses in the presence or absence of endothelium. Endothelial cells are, therefore, not required for the mediation of hypoxic pulmonary vasoconstriction.** [P.S.E.B.M. 1992, Vol 201]

When the oxygen tension decreases in a region of the lung, the small pulmonary arteries constrict locally. This hypoxic pulmonary vasoconstriction (HPV) is unique to the lung and is the principal mechanism actively regulating local ventilation/perfusion ratios. HPV contributes importantly to homeostasis by improving pulmonary gas exchange, and to pathophysiology by increasing pulmonary vascular resistance.

The mechanism underlying HPV remains unknown, but the most frequent suggestion has been that HPV depends upon the release of a mediator from some cell other than the vascular smooth muscle. Many mediators and other lung cells have been excluded experimentally, but recently, several investigators have proposed that an intact endothelium is essential for HPV and there are two hypotheses; in one, hypoxia causes the reduced production of a dilator, and, in the other it causes an increased release of a constrictor. The uncertainty in this field is well illustrated by two recent reviews (1, 2). The present work has demonstrated definitively that endothelium is not essential for HPV; therefore, the search for the elusive mechanism can focus on the vascular smooth muscle cell itself.

Method

Female cats (2.5–3.5 kg) were anesthetized with 50 mg (1 ml) of Ketalar (ketamine hydrochloride) intramuscularly plus 30 $mgs.kg^{-1}$ of sodium pentobarbital intraperitoneally, in accordance with the Institutional Animal Care Committee's guidelines, and exsanguinated by severing the carotid arteries. The heart and lungs were removed into iced Hanks' buffered salt solution (pH 7.4). The lungs were separated into the individual lobes, and, with the aid of a dissecting microscope, the intrapulmonary arteries were dissected free of surrounding tissue and vessel diameters were measured using a calibrated reticle. These vessels were placed in labeled vials containing Hanks' balanced salt solution and maintained at 4°C.

The isolated vessels were cut into appropriate lengths and measured. Vessel segments adjacent to each other were used as control (with endothelium) or for experiments (no endothelium). The endothelium was removed by placing one tine of a serrated hemostat in the lumen of the vessel, which was then gently rotated back and forth 10 times on wet Whatman filter paper. Two wires were threaded through the lumen of the vessel. One wire was fixed to an L-shaped support and the other wire was tied to a Grass force displacement transducer by a length of 6-0 silk. The suspended vessel was placed in a heated muscle bath containing 15 ml of Earle's balanced salt solution bubbled with a nor-

[1] To whom requests for reprints should be addressed at Center for Anesthesia Research, Hospital of the University of Pennsylvania, 3400 Spruce Street, 7 Dulles Building, Philadelphia, PA 19104.

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moxic gas mixture (30% oxygen, 5% carbon dioxide, balance nitrogen).

The vessels were stretched to a tension of 500 mg, selected on the basis of preliminary experiments, and then allowed to equilibrate for 60 min at constant tension. At the end of the 60 min, the baths were emptied and refilled with warm, gassed Earle's salt solution (0.15 ml). Norepinephrine was added to produce a concentration in the bath of $1*10^{-6}$ M; seven minutes into this response, 0.15 ml of acetylcholine was added to the bath ($1*10^{-6}$ M). After 5 min, the muscle preparation was washed and fresh, gassed Earle's solution was added and phenylephrine was added to the muscle bath ($2.45*10^{-6}$ M). The vessels were stimulated with an hypoxic gas mixture (0% oxygen, 5% carbon dioxide, balance nitrogen) for 20 min, followed by 20 min of the normoxic gas mixture. The intermittent hypoxic//normoxic gas challenges were repeated five times. Ten minutes into the final hypoxic challenge, acetylcholine was again administered to the muscle bath ($1*10^{-6}$ M).

Measurements were made of the increasing or decreasing isometric tension by means of a Grass force displacement transducer (model no. FT03C) and the results were recorded on a Kipp and Zonen recorder (BD101). The oxygen tension of the solution bathing the vessels was measured by an Harvard oxygen analyzer (model no. 102), and the temperature was maintained at a constant 37°C. The data are expressed as mean ± SE and were analyzed by repeated-measures analysis of variance, and the statistical differences among means were determined by Tukey's tests, where $P \leq 0.05$ was considered significant.

Results

The mean weight of the cats was 3.19 ± 0.5 kg. With and without endothelium, the lengths of the vessels were 2.3 ± 0.6 mm and 2.3 ± 0.5 mm and the diameters were 0.87 ± 0.06 mm and 0.86 ± 0.06 mm, respectively. The oxygen tension in the vessel baths during normoxia was 208 ± 10 mm Hg and during hypoxia was 26.2 ± 0.6 mm Hg. The baseline tension after injection of phenylephrine and before the first hypoxic challenge was 566 ± 53 mg with endothelium and 582 ± 52 mg without endothelium. These values did not change significantly with time. The changes in tension with additions of drugs or hypoxia are summarized in Table I. The response to acetylcholine at the beginning and the end was dilator (i.e., reduction of tension) when endothelium was present and constrictor when endothelium was absent (Fig. 1). There were no differences in the response to norepinephrine nor the constrictor response to hypoxia in the presence or absence of endothelium, and the hypoxic response did not change with repetition after the first challenge and was well sustained, as demonstrated by the small differences between the hypoxic responses measured at 10 min and 20 min.

Table I. Tension Changes in Feline Pulmonary Arteries in Response to Hypoxia and Other Agonists with and without Endothelium[a]

	Endothelium	
	With (tension mg)	Without (tension mg)
Norepinephrine	308 ± 57	423 ± 80
Acetylcholine	−216 ± 58	552 ± 113
First hypoxia		
10 min	198 ± 48	231 ± 24
20 min	167 ± 47	218 ± 31
Second hypoxia		
10 min	372 ± 94	323 ± 53
20 min	316 ± 90	285 ± 53
Third hypoxia		
10 min	417 ± 124	416 ± 78
20 min	381 ± 109	388 ± 73
Fourth hypoxia		
10 min	452 ± 125	447 ± 93
20 min	431 ± 129	418 ± 76
Fifth hypoxia		
10 min	512 ± 135	485 ± 103
20 min	495 ± 138	468 ± 89
Acetylcholine	−306 ± 87	994 ± 24

[a] Values for mean ± SE are shown and $n = 9$. Positive values are constrictor and negative values are dilator.

Discussion

The presence or absence of endothelial cells was confirmed functionally by the method of Furchgott and Zawadzki (3); accordingly, the response of the norepinephrine-preconstricted vessels to acetylcholine was dilator when endothelium was present and constrictor after the endothelium had been removed by mechanical rubbing. These intrapulmonary cat arteries constricted reversibly, consistently, and repeatedly to hypoxia to the same extent in the presence and absence of endothelial cells. Endothelial cells are, therefore, not required for hypoxic pulmonary vasoconstriction (HPV).

The recent emphasis on the endothelial cell as the origin of the mediator for the HPV response is based on its intimate relationship to the vascular smooth muscle and on the recognition that endothelial cells synthesize and release a number of vasoactive materials (4–8). Many of these have been suggested as possible mediators for HPV, but on theoretical and experimental grounds, none has been entirely convincing.

Several investigators have proposed that a low oxygen tension inhibits the synthesis of endothelium-derived relaxing factor (EDRF), which results in an increase in vascular tone. The action of EDRF is to induce vascular smooth muscle dilation by activating guanylate cyclase and the synthesis of cGMP. This activation is inhibited by methylene blue. In isolated rat lung

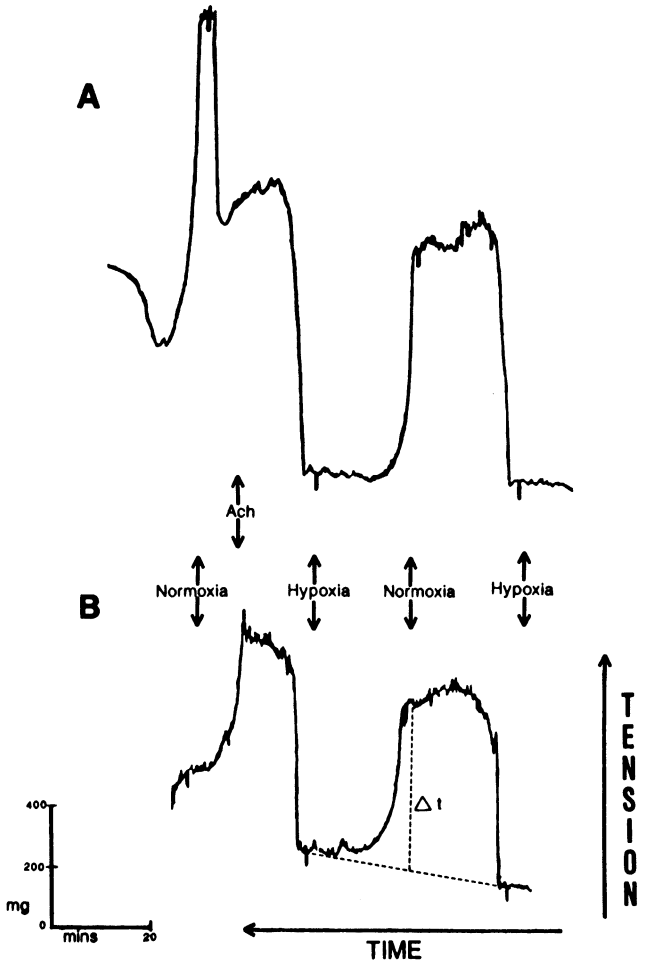

Figure 1. Experimental records of the tension changes in two pulmonary vessels for the fourth and fifth 20-min hypoxic responses. Tracing (B) is from a vessel with an endothelium, and tracing (A) is from a vessel without endothelium. The tracing should be read from right to left. The arrows marked hypoxia, normoxia, and Ach (acetylcholine) indicate when the gases are changed and acetylcholine is given, respectively. The derivation of the data for one response at 20 min is illustrated with a dashed line drawn connecting the baseline at the start of the hypoxic challenges; (Δt) represents the tension difference measured after 20 min of hypoxia and just prior to the onset of the normoxic gas phase. Acetylcholine action is shown as a (A) constrictor in and (B) dilator in, yet the HPV response was similar in both vessels.

preparations, two independent laboratories reported that the addition of sufficient methylene blue to the perfusate to cause a constrictor response to acetylcholine demonstrated inhibition of EDRF, but potentiated the response to hypoxia (9, 10). Hyman *et al.* (11), using methylene blue *in vivo* in the whole animal, found an increase in the basal tone of the pulmonary vessels and a reduced responsiveness to hypoxia; however, the EDRF activity was incompletely blocked in this preparation. Johns *et al.* (12) observed that in the presence of hypoxia, the cGMP content of rabbit pulmonary vascular smooth muscle decreased and there was a decrease in the dilator ability of the vessels. However, the hypoxic response reported by this group was only transiently constrictor, even in the control vessels, and, therefore, atypical. On balance, these indirect studies do not support the concept that reduction of EDRF is the mediator of HPV, and the basis for the differences reported probably rests on the uncertain specificity of the blocker (i.e., methylene blue). The speculation that the mediator for HPV might be release of a constrictor from the endothelium (i.e., endothelium-derived constricting factors or endothelin) is not supported by data or the known characteristics of these animals.

Rodman *et al.* (13) observed in rat extrapulmonary arteries that the hypoxic response was reduced in vessels denuded of endothelium. They concluded that hypoxic constriction is caused largely by decreased EDRF activity. Similarly, Holden and McCall (14), in experiments in which endothelial cells were removed from the larger pulmonary arteries of the pig, observed that the response to hypoxia decreased significantly. In both these preparations, physical trauma associated with removal of the endothelium may have reduced vascular responsiveness either by release of vasodilator materials or by injury to the contractile apparatus. This conclusion is supported by the failure of the HPV response to return when endothelial cells were added back to the porcine pulmonary arteries in the study by Holden and McCall (14) and the demonstration by Cosmi *et al.* (15) of light and electron microscopic evidence of damage to rat pulmonary arteries after mechanical removal of the endothelium. We have observed hypoxic constrictor responses in preconstricted (e.g., with phenylephrine in the present work) pulmonary arteries of all sizes. This tone dependence may be the basis for some previous reports of failure to observe hypoxic constriction in relaxed pulmonary vessels larger than 0.5 mm (16).

In conclusion, the present study has shown that endothelial cells are not required for HPV in isolated pulmonary vessels from adult cats, and supports the recent reports of hypoxic constriction in isolated endothelium-denuded pulmonary vessels from calf (17) and rat (18) lungs and in pure cultures of fetal bovine pulmonary vascular smooth muscle cells (19). The inconsistencies in previous reports are probably the result of changes in the responsiveness of the vasculature caused by trauma or by modulating influences of drugs and other materials, including the products of endothelial cells.

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1. Fishman AP (Ed). The enigma of hypoxic pulmonary vasoconstriction. In: The Pulmonary Circulation: Normal and Abnormal

Mechanisms, Management and the National Registry. University of Pennsylvania Press, Ch 9, pp109–129, 1990.
2. Rodman DM, Voelkel NF. Regulation of vascular tone. In: Crystal RG, West JB, Eds. The Lung: Scientific Foundation. New York: Raven Press, Vol 1, Ch 5.2.1.3, 1991.
3. Furchgott RF, Zawadzki J. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature **288**:373–376, 1980.
4. Vane JR, Gryglewski RJ, Botting RM. The endothelial cell as a metabolic and endocrine organ. TIPS **8**:491–496, 1987.
5. Levin RI, Weksler BB, Marcus AJ, Jaffe EA. Prostacyclin production by endothelial cells. In: Jaffe EA, Ed. Biology of Endothelial Cells. Ch 24, pp228–247, 1984.
6. Furchgott RF. Role of endothelium in responses of vascular smooth muscle. Circ Res **53**:557–573, 1983.
7. Rubanyi GM, Vanhoutte PM. Hypoxia releases vasoconstrictor substance from the canine vascular endothelium. J Physiol **364**:45–56, 1985.
8. Komori K, Lorenz RR, Vanhoutte PM. Nitric oxide, AcH, and electrical and mechanical properties of canine arterial smooth muscle. Am J Physiol **255**:H207–H212, 1985.
9. Marshall C, Marshall BE. Endothelium derived relaxing factor is not responsible for inhibition of hypoxic pulmonary vasoconstriction by inhalational anesthetics. Anesthesiology **73**:441–448, 1990.
10. Mazmanian GM, Baudet B, Brink C, Cerrina J, Kirkiacharian S, Weiss M. Methylene blue potentiates vascular reactivity in isolated rat lungs. J Appl Physiol **66**:1040–1045, 1989.
11. Hyman AL, Lipton HL, Kadowitz PJ. Methylene blue prevents hypoxic pulmonary vasoconstriction in cats. Am J Physiol **260**:H586–H592, 1991.
12. Johns RA, Linden JM, Peach MJ. Endothelium-dependent relaxation and cyclic GMP accumulation in rabbit pulmonary artery are selectively impaired by moderate hypoxia. Circ Res **65**:1508–1515, 1989.
13. Rodman DM, Yamaguchi T, Hasunuma K, O'Brien RF, McMurty IF. Effects of hypoxia on endothelium-dependent relaxation of rat pulmonary artery. Am J Physiol **258**:L207, 1990.
14. Holden WE, McCall E. Hypoxia-induced contractions of porcine pulmonary artery strips depend on intact endothelium. Exp Lung Res **7**:101, 1984.
15. Cosmi S, Dollahon N, Gardner R, Marshall C. A microscopic evaluation of endothelial cell removal techniques as applied to small intrapulmonary arteries. FASEB J **4**:A909, 1990.
16. Madden JA, Dawson CA, Harder DR. Hypoxia-induced activation in small isolated pulmonary arteries from the cat. J Appl Physiol **59**:113–118, 1985.
17. Burke-Wolin T, Wolin MS. H_2O_2 and cGMP may function as an oxygen sensor in the pulmonary artery. J Appl Physiol **66**:167–170, 1989.
18. Yuan XJ, Tod ML, Rubin LJ, Blaustein MP. Contrasting effects of hypoxia on tension in rat pulmonary and mesenteric arteries. Am J Physiol **259**:H281–H289, 1990.
19. Murray TR, Chen L, Marshall BE, Macarak EJ. Hypoxic contraction of cultured pulmonary vascular smooth muscle cells. Am J Resp Cell Mol Biol **5**:457–465, 1990.