ROLE OF ENDOTHELIUM IN BIPHASIC HYPOXIC RESPONSE OF THE ISOLATED PULMONARY ARTERY IN THE RAT

YUJI OGAWA, M.D., JUN-ICHI KAWABE, M.D., SOKICHI ONODERA, M.D.
KATSUYUKI TOBISE, M.D., KAZUTOYO MORITA, M.D.
TAKAYUKI HARADA, M.D., TOMOYA HIRAYAMA, M.D.
AND AKINORI TAKEDA, M.D.

We investigated the roles of the endothelium in the hypoxic responses of the isolated main pulmonary artery (PA) in the rat. Hypoxia was induced by gassing an organ chamber with 95% N₂+5% CO₂ (PO₂=34.6±3.1 Torr) instead of 16% O₂+5% CO₂+balance N₂ (PO₂=92.8±3.0 Torr). Vascular rings were precontracted with 2×10⁻⁸ M phenylephrine. A transient hypoxic contraction and a subsequent relaxation were observed in the endothelium-intact rings. The hypoxic contraction was reduced in the endothelium-denuded rings. In contrast, there were no significant differences between the hypoxic relaxation in the endothelium-intact and endothelium-denuded rings. Inhibitors of endothelium-derived relaxing factor (EDRF) activity, 2×10⁻⁶ M NG-monomethyl-L-arginine (L-NMMA) and 10⁻⁶ M methylene blue, produced 53% and 66% reductions in hypoxic contraction, respectively. Furthermore, the amount of cyclic GMP in the endothelium-intact PA rings which had been precontracted with phenylephrine decreased from 2.10±0.45 pmol/mg protein during normoxia to 0.90±0.18 pmol/mg protein during hypoxia. Indomethacin and OKY-046 did not influence hypoxic contraction or relaxation. These results suggest that hypoxic contraction of the isolated pulmonary artery in the rat is partially induced by inhibition of the release of EDRF. (*Jpn Circ J* 1993; **58**: 228–236)

HYPOXIC pulmonary vasoconstriction (HPV) is a regulatory mechanism which matches local lung perfusion with ventilation,¹ however, its precise mechanism is unknown. The primary site of HPV is believed to be in the precapillary arteries (diameter<300 μm).^{2,3} In addition, several investigators have demonstrated that hypoxic contraction is also observed in isolated large pulmonary artery.^{4,5} Recent reports have shown that endothelium-derived relaxing factor (EDRF) plays an important role in the regulation of vascular tone,⁶ and that it is

nitric oxide (NO) or a similar nitrogen-based radical species.⁷ In earlier studies, De Mey and Vanhoutte⁸ and Holden and McCall⁴ investigated the role of the pulmonary endothelium in sensing hypoxia using a ring preparation of the pulmonary artery. They demonstrated that hypoxic contraction depended upon the presence of functioning endothelium. However, it is still unknown whether hypoxic contraction is due to the interruption of the production of EDRF or to the generation of endothelium-derived contracting factor (EDCF). Recently, Johns et al⁹ and Rodman et al¹⁰ indicated that inhibitors of EDRF activity (hemoglobin, methylene blue and hydroquinone) atten-

Key words:

Endothelium-derived relaxing factor
Pulmonary artery

(Received February 28, 1992; accepted August 12, 1992)

First Department of Internal Medicine, Asahikawa Medical College Nishikagura, 4-5-3-11, Asahikawa 078, Japan

Mailing address: Yuji Ogawa, M.D., First Department of Internal Medicine, Asahikawa Medical College, Nishikagura, 4-5-3-11, Asahikawa 078, Japan

Fig.1. Typical traces of the response to hypoxia in the endothelium-intact PA rings. Resting ring received no agonist (a). Rings were precontracted with 2×10^{-8} M phenylephrine (b), 5×10^{-6} M 5-HT (c), or 20 mM KCl (d). Hypoxic contraction was not observed in the resting rings. However, a transient hypoxic contraction and a subsequent relaxation were observed in the precontracted rings. (e) shows the time course of the phenylephrine-induced contraction.

uated the hypoxic contraction of isolated PA, and that hypoxic pulmonary contraction was caused in part by decreased EDRF activity. On the other hand, other investigators have indicated that inhibitors of EDRF activity enhanced HPV, and that EDRF tended to counteract HPV.¹¹⁻¹³ These different results varied with the species or experimental methods. However, even in the same preparation (isolated rat pulmonary artery), Archer et al reported that NG-monomethyl-L-arginine (L-NMMA) en-

hanced hypoxic contraction.¹²

The purpose of the present experiments was to clarify the role of the endothelium in the hypoxic response of isolated rat pulmonary artery, by evaluating the effects of a specific EDRF inhibitor, L-NMMA,¹⁴ and by measuring the accumulation of cyclic GMP in the pulmonary arterial rings. We also investigated the role of eicosanoids in the hypoxic contraction and relaxation responses.

MATERIALS AND METHODS

The experiments were performed on the rings of the left main pulmonary arteries from male Sprague-Dawley rats (Charles River Japan Inc. Japan), 8–12 weeks old, weighing an average of 331 g. Rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). The chests were opened, the hearts and lungs were removed en bloc and placed in Krebs-Henseleit solution with the following composition: NaCl 118.1, KCl 4.7, CaCl_2 2.5, KH_2PO_4 1.2, MgSO_4 1.2, NaHCO_3 25.0, and glucose 11.0. The left main pulmonary arteries were then carefully excised. After the removal of connective tissue, these vessels were cut into ring segments 5 mm long. In some rings, the endothelium was denuded by inserting a teflon tube into the lumen. Endothelium-intact vessels were also used. The rings were then mounted in water-jacketed baths containing Krebs-Henseleit solution at 37°C, gassed with a mixture of 16% O_2 + 5% CO_2 + balance N_2 to produce a PO_2 of 92.8 ± 3.0 Torr and a pH of 7.3–7.4. The upper end of the ring was connected to the lever of a force displacement transducer (TB611T, TB612T, Nihon Koden, Tokyo) by a silk thread. Resting tension for the rings was maintained at 1 g throughout the experiment. Isometric contraction was recorded on an XY recorder (D72-BP, SP-H3C, Riken Denshi, Tokyo). The rings were left to equilibrate for 2 h in the bath solution before the start of the experiments. The functional status of the endothelium was ascertained by measuring the relaxant effects of acetylcholine (ACh) after precontraction with 5×10^{-7} M phenylephrine. To produce hypoxia, the gas mixture was changed to 5% CO_2 + 95% N_2 to create a PO_2 of 34.6 ± 3.1

TABLE I THE HYPOXIC RESPONSES IN THE RAT PULMONARY ARTERIAL RINGS PRECONTRACTED WITH PHENYLEPHRINE (PHE), 5-HT OR KCL

	<i>n</i>	<i>precontraction</i> (% of a maximum contraction)	<i>hypoxic contraction</i>	<i>hypoxic relaxation</i>
<i>Phe</i> 2×10^{-8} M	9	144.6 ± 15.7 mg (39.1%)	87.2 ± 10.2 mg* (23.7%)	-85.7 ± 35.7 mg
<i>5-HT</i> 5×10^{-6} M	6	141.7 ± 24.7 mg (38.5%)	108.8 ± 7.0 mg** (35.7%)	-91.7 ± 18.2 mg
<i>KCl</i> 20 mM	7	151.0 ± 25.3 mg (35.6%)	42.9 ± 6.4 mg (10.1%)	-119.2 ± 24.5 mg
<i>Phe</i> 2×10^{-8} M + 10^{-5} M indomethacin	7	110.3 ± 18.3 mg (30.0%)	96.5 ± 12.6 mg (26.2%)	-79.0 ± 10.8 mg
<i>Phe</i> 2×10^{-8} M + 10^{-5} M OKY-046	7	152.3 ± 30.9 mg (41.3%)	89.7 ± 18.4 mg (24.5%)	-92.0 ± 15.6 mg

There was no significant difference between the precontraction or hypoxic relaxation among the 3 agonists. In contrast, the hypoxic contraction in the rings which had been precontracted with phenylephrine and 5-HT was greater than that in the rings which had been precontracted with KCl (* $p < 0.05$, ** $p < 0.01$ compared with KCl). Indomethacin (10^{-5} M) and OKY-046 (10^{-5} M) did not affect the hypoxic response. Precontraction is expressed as a percentage of the respective maximum agonist-induced contraction, respectively. Endothelium was intact. *n*=Number of rings. Data are given as means $\pm$ SE.

Torr and a pH of 7.3–7.4. The oxygen pressure decreased from 92.8 ± 3.0 Torr to 34.6 ± 3.1 Torr in about 60 sec. The oxygen tension was measured by a pH/Blood Gas Analyzer 813 (Instrumentation Laboratory).

Hypoxic response protocol

Resting rings and rings which had been precontracted with 2×10^{-8} M phenylephrine, 5×10^{-6} M 5-hydroxytryptamine (5-HT), or 20 mM KCl were tested for hypoxic response. After a steady-state was achieved, normoxic gas was exchanged for anoxic gas and the delta tension was measured. In the rings which had been precontracted with vasoconstrictive agents, the baseline level was the point at which the contraction became steady-state. The hypoxic challenges were repeated until the responses became constant ($\pm 5\%$ difference from the previous response). The hypoxic responses were tested in the endothelium-intact and endothelium-denuded rings, and the effects of various inhibitors were observed. We used L-NMMA (2×10^{-6} M, 3×10^{-5} M) or methylene blue (10^{-6} M) to inhibit EDRF activity. The dose-response for L-NMMA in this preparation was also examined. The effects of indomethacin (cyclooxygenase inhibitor, 10^{-5} M) and OKY-046 (thromboxane synthase inhibitor, 10^{-5} M) on the

hypoxic response were also tested. The specimens were incubated with the inhibitors for 30 min before the addition of phenylephrine. The ACh-induced vasorelaxation was evaluated by the EC₅₀ and as a percentage of the maximal contraction caused by 5×10^{-7} M phenylephrine.

Hypoxia and Cyclic GMP

The amount of cyclic GMP in the endothelium-intact rings which had been precontracted with 2×10^{-8} M phenylephrine was determined during normoxia and hypoxia. The maximal contraction was observed when the rings were exposed to anoxic gas for about 60–90 seconds. At the point of the maximal contraction, the organ baths were dropped and the rings were rapidly frozen by liquid nitrogen. Control normoxic rings were frozen during normoxic gas exposure at a similar point. Cyclic GMP was extracted by homogenizing the frozen rings in 0.5 ml of iced 6% trichloroacetic acid and centrifuging the homogenate at 3000 rpm for 15 min. Extraction was then performed in triplicate with 3 ml of diethyl ether. The ether was then removed by evaporation at 70 °C. Measurements were made in duplicate on 100- μ l aliquots with a radioimmunoassay kit (Yamasa-shoyu, Tokyo).

Fig.2. Typical traces of the response to hypoxia in the endothelium-intact (a) and endothelium-denuded (b) PA rings. In the endothelium-denuded ring, ACh-induced vasorelaxation and hypoxic contraction were not observed.

Fig.3. Hypoxic contraction in the endothelium-intact (END (+)) and endothelium-denuded (END (-)) rings. Open bars indicate the 2×10^{-8} M phenylephrine-induced contraction. Closed bars indicate the developed hypoxic contraction. Phenylephrine-induced contraction in the endothelium-denuded rings was greater than that in the endothelium-intact rings. Hypoxic contraction was markedly decreased in the endothelium-denuded rings. (* $p < 0.05$) $n=5$, Data are given as means $\pm$ SE.

Reagents

Standard reagents were obtained from Sigma Chemical Company (St. Louis, MO). L-NMMA was obtained from Calbiochem Corporation (La Jolla, CA). OKY-046 was a generous gift from Ono Pharmaceutical, Tokyo, Japan. All reagents, except for indomethacin, were dissolved with distilled and deionized water. Indomethacin was dissolved in alcohol and distilled water such that the maximum bath concentration was $< 0.01\%$.

Data Analysis

Results were expressed as means $\pm$ SE. Statistical analyses were performed using a one way analysis of variance (ANOVA) followed by the Dunnett test. P values 0.05 were considered to be statistically significant.

RESULTS

PA ring hypoxic response

Neither endothelium-intact nor endothelium-denuded resting PA rings which had been exposed to hypoxic conditions developed tension. In the resting rings, acetylcholine did not produce endothelium-dependent relaxation. In contrast, a transient contraction and a subsequent relaxation were observed in the endothelium-intact rings which had been precontracted with

Fig.4. Dose-response curve for L-NMMA in the isolated rat PA. Endothelium was intact. $n=8$, Data are given as means $\pm$ SE.

vasoconstrictive agents: i.e., phenylephrine, 5-HT or KC. Reoxygenation returned the tension to its level in the normoxic condition (Fig. 1). The tensions developed by 2×10^{-8} M phenylephrine, 5×10^{-6} M 5-HT, and 20 mM KCl, and the hypoxia-induced developed tension are presented in Table I. At these concentrations, the 3 vasoactive agents produced similar amounts of force in the normoxic condition. Although the hypoxic contraction in the rings which had been precontracted with phenylephrine and 5-HT was significantly greater than that in the rings which had been precontracted with KCl, there was no difference between the hypoxic contraction in the rings which had been precontracted with phenylephrine and those which had been precontracted with 5-HT. There were no significant differences between the hypoxic relaxation among the 3 vasoconstrictive agents (Table I). The hypoxic contraction occurred at a PO_2 of 50–60 Torr. The contractile tone induced by phenylephrine gradually decreased, however, the hypoxic relaxation occurred faster than the loss of the tone which had been induced by phenylephrine (Fig. 1).

In the rings which had been precontracted with 2×10^{-8} M phenylephrine, the hypoxia-induced developed tension was 87.2 ± 10.2 mg. In contrast, in the endothelium-denuded rings, the delta tension was reduced (6.2 ± 4.1 mg) (Figs. 2, 3). There was no difference between the hypoxic relaxation in the endothelium-intact and endothelium-denuded rings.

Fig.5. The effects of L-NMMA on the hypoxic contraction of the isolated rat PA. Open bars indicate the 2×10^{-8} M phenylephrine-induced contraction, and closed bars indicate the developed hypoxic contraction. Although L-NMMA enhanced the phenylephrine-induced contraction, it attenuated the hypoxic contraction (* $p < 0.01$). The hypoxic contraction was not reduced in the rings which had been precontracted with 10^{-7} M phenylephrine, which had a developed tension similar to that of rings which had been precontracted with 2×10^{-8} M phenylephrine plus 2×10^{-6} M L-NMMA. Endothelium was intact. $n=9$, Data are given as means $\pm$ SE.

The inhibitory effects of L-NMMA were tested in the endothelium-intact rings which had been precontracted with 2×10^{-8} M phenylephrine. L-NMMA produced dose-dependent contractile response (Fig. 4). Therefore, we used a relatively low dose of L-NMMA, 2×10^{-6} M, which did not significantly affect the basal tension. This dose of L-NMMA reduced the ACh-induced vasorelaxation: EC_{50} went from 2.0×10^{-8}

Fig. 6. The effects of 10^{-6} M methylene blue (MB) on the hypoxic contraction of the isolated rat PA. Open bars indicate the 2×10^{-8} M phenylephrine-induced contraction and closed bars indicate the developed hypoxic contraction. Although methylene blue, 10^{-6} M, enhanced the phenylephrine-induced contraction, it attenuated the hypoxic contraction ($*p < 0.05$). Endothelium was intact. $n = 6$, Data are given as means $\pm$ SE.

M to 1.2×10^{-7} M, and the maximal relaxation fell from 74% to 39% ($p < 0.01$). Although the above dose of L-NMMA enhanced the phenylephrine-induced contraction, it reduced the hypoxic contraction from 78.1 ± 11.4 mg to 41.1 ± 13.2 mg (53%, $p < 0.01$). However, the hypoxic contraction may have been reduced outwardly because the phenylephrine-induced contraction was enhanced. To exclude this possibility, the hypoxic response was tested in rings which had been precontracted with 10^{-7} M phenylephrine and with a developed tension similar to that of rings which had been treated with 2×10^{-8} M phenylephrine plus 2×10^{-6} M L-NMMA. The hypoxic contraction of the rings which had been precontracted with 10^{-7} M phenylephrine was not reduced (Fig. 5). We also tested the effects of a relatively high dose of L-NMMA. L-NMMA at 3×10^{-5} M produced contraction (54.3 ± 13.1 mg, $n = 9$) in this vessel. In the presence of 3×10^{-5} M L-

Fig. 7. The amount of cyclic GMP in the endothelium-intact rat PA during normoxia and hypoxia. The amount of cGMP was significantly reduced by hypoxia. ($*p < 0.01$) $n = 8$, Data are given as means $\pm$ SE.

NMMA, the phenylephrine-induced contraction increased from 125.8 ± 15.7 mg to 293.8 ± 21.2 mg, and the hypoxic contraction fell from 94.2 ± 13.9 mg to 64.0 ± 10.4 mg (77.5%, $p < 0.01$). Methylene blue, 10^{-6} M, also reduced the hypoxic contraction from 113.0 ± 63.0 mg to 57.0 ± 47.0 mg (Fig. 6). The effects of 10^{-5} M indomethacin or 10^{-5} M OKY-046 were tested in 7 paired endothelium-intact rings. There was no significant difference between the hypoxic contraction and hypoxic relaxation in the control and the indomethacin or OKY-046-treated rings (Table I).

Cyclic GMP and hypoxia

Hypoxia reduced the amount of cyclic GMP in the endothelium-intact rings from 2.10 ± 0.46 pmol/mg protein to 0.90 ± 0.18 pmol/mg protein (Fig. 7).

DISCUSSION

The primary site of hypoxic pulmonary vasoconstriction (HPV) has been thought to be the precapillary vessels. However, some investigators have indicated that hypoxic pulmonary contraction has also been observed in isolated large pulmonary arteries^{4,5}. Therefore, we examined the mechanism of HPV by using isolated pulmonary arteries.

In the present study, hypoxic contraction was observed in endothelium-intact rings which had been precontracted with vasoconstrictors, but not in the unstimulated rings. Earlier reports indicated that hypoxic contraction was observed in resting rings of the cat² and rat⁵, and both vessels showed a spontaneous contraction or a small amount of basal activation. In our study, ACh-induced endothelium-dependent relaxation was not observed in the resting ring. An increased basal level of smooth muscle activation may contribute to the hypoxic contraction in resting rings⁵.

Recent studies have shown that the endothelium plays an important role in the regulation of vascular tone⁶. In the PA from the pig⁴, rat⁵ and dog⁸, hypoxic contraction was reduced by removal of the endothelium. In the present study, the hypoxic contraction of the endothelium-denuded rings was also reduced markedly. These data suggest that the endothelium might play an important role in hypoxic contraction. An earlier report indicated that endothelium-dependent relaxation caused by ACh was eliminated under anoxic conditions¹⁵. This finding suggested that either the release of EDRF was reduced or the activity of EDRF was attenuated under hypoxic conditions and that these mechanisms were related to HPV.

In our study, we tested the effects of chemical EDRF (NO) inhibitors on the response to hypoxia. L-NMMA (a specific inhibitor of the production of NO) and methylene blue (a soluble guanylate cyclase inhibitor) were used. These inhibitors caused a dose-dependent contraction in the isolated rat PA^{10,12}. In the present experiment, L-NMMA caused a dose-dependent contractile response. Therefore, we used a relatively low dose of the drugs which inhibited ACh-induced relaxation but did not affect basal tension, because an elevated tone induced by a high dose of L-NMMA was thought to affect the responses to hypoxia⁵. Both L-NMMA and methylene blue enhanced the phenylephrine-induced contraction, probably because these drugs inhibited the EDRF activity which was enhanced by the elevated tone¹⁶. The hypoxic contraction was reduced in the presence of the EDRF inhibitors. Therefore, the effect of the suppression of the EDRF activity,

i.e., the hypoxic contraction, may have been attenuated because the EDRF activity had already been reduced. The reduction in hypoxic contraction could not be explained simply by the increase in normoxic force produced by chemical inhibition of EDRF because the hypoxic contraction associated with the increase in force produced by 10^{-7} M phenylephrine, which had a developed tension similar to that of rings which had been precontracted with 2×10^{-8} M phenylephrine plus 2×10^{-6} M L-NMMA, was not similarly attenuated. The magnitude of the hypoxic contraction in the rings which had been precontracted with phenylephrine or 5-HT was significantly greater than that in the rings which had been precontracted with KCl. The reason for this difference is unknown, however, phenylephrine and 5-HT have been reported to stimulate the release of EDRF by activating the α_1 -adrenoceptor¹⁷ and the 5-HT₁-like receptor¹⁸, respectively, in endothelial cells. The production of EDRF in rings which have been precontracted with phenylephrine or 5-HT may be higher than that in rings which have been precontracted with KCl. If so, the effect of suppression of EDRF activity would be greater in rings which have been precontracted with phenylephrine or 5-HT than in those precontracted with KCl. Furthermore, Ca^{2+} release from intracellular storage sites is reportedly involved in the mechanism of hypoxic contraction in isolated human pulmonary artery¹⁹. Hypoxic contraction in rings which have been precontracted with receptor agonists is thought to be greater than that in those which have been precontracted with KCl.

Cyclic GMP is the second messenger of the EDRF/NO dependent relaxation, and its concentration reflects the activity of EDRF. In our study, the accumulation of cyclic GMP in the endothelium-intact ring was reduced by hypoxic stimulation. These results agreed with earlier reports^{9,10} and were consistent with the hypothesis that the attenuation of EDRF activity is one of the mechanisms of hypoxic contraction. The precise mechanism of the hypoxia-induced inhibition of EDRF activity is not clear. High oxygen tension reportedly destroys EDRF as a result of the generation of the superoxide radical, however, hypoxia could

not be due to an effect on EDRF once produced²⁰ Recently, Rengasamy and Johns reported that hypoxia inhibited EDRF/NO synthase activity²¹

On the other hand, some investigators have indicated that hypoxic contraction was generally enhanced by the inhibition of EDRF, in experiments using perfused rat lung¹¹⁻¹³ These different effects of EDRF inhibitors on hypoxic pulmonary contraction can not be explained by the differences between the species studied. The size of the vessel and the experimental methods may be important factors. Furthermore, NO synthesis may be enhanced secondarily by the elevated tone.

Other mechanisms of hypoxic contraction which involved the endothelium were also considered. Wadsworth et al. indicated that a decrease in prostacyclin enhanced hypoxic contraction in sheep coronary artery²² Biaggioni et al. reported that adenosine, the tissue levels of which increase during hypoxia, produced pulmonary vasoconstriction in sheep by the generation of a thromboxane/endoperoxide product²³ However, the effects of a cyclooxygenase inhibitor or thromboxane synthase inhibitor on hypoxic contraction or relaxation were not observed in the present study. The hypoxic response in isolated rat PA does not appear to be mediated by the production of prostanooids. Another possible mechanism is the release of endothelium-derived contracting factor (EDCF). Rubanyi and Vanhoutte indicated that hypoxia and anoxia cause a release of diffusible vasoconstrictor substances from endothelial cells in isolated canine coronary artery²⁴ The EDCF produced by hypoxia has been referred to as EDCF₁²⁵ A vasoconstricting peptide, endothelin, has recently been isolated from cultured porcine endothelial cells²⁶ However, endothelin is not believed to be a mediator of hypoxic vasoconstriction because endothelin release is not elevated by hypoxia, and because its effects are long-lasting, in contrast to the rapid onset of, and recovery from hypoxic contraction²⁷⁻²⁹

Hypoxic relaxation did not depend on the endothelium in the present study. Relaxation may be due to an inhibitory effect of hypoxia on the contractile response. Recently, Daut et al. reported that the hypoxic

dilatation of guinea pig coronary artery was mediated by ATP-sensitive potassium channels³⁰

In summary, our data indicate that hypoxic contraction of the isolated rat PA is in part induced by a decrease in the release of EDRF. Although these results were acquired from isolated large pulmonary arteries which were precontracted with vasoconstrictor agents, Ignarro et al. indicated that the production of EDRF in the smaller vessels was more than that in the large vessels⁶ This mechanism may be present under hypoxic conditions in vivo.

REFERENCES

1. VON EULER US, LILJESTRAND G: Observations on the pulmonary arterial blood pressure in the cat. *Acta Physiol Scand* 1946; **12**: 301-320
2. MADDEN JA, DAWSON CA, HARDER DR: Hypoxia-induced activation in small isolated pulmonary arteries from the cat. *J Appl Physiol* 1985; **59**: 113-118
3. SHIRAI M, SADA K, NINOMIYA I: Effects of regional alveolar hypoxia and hypercapnia on small pulmonary vessels in cats. *J Appl Physiol* 1986; **61**: 440-448
4. HOLDEN WE, MCCALL E: Hypoxia-induced contractions of porcine pulmonary artery strips depend on intact endothelium. *Exp Lung Res* 1984; **7**: 101-112
5. RODMAN DM, YAMAGUCHI T, O'BRIEN RF, MCMURTRY IF: Hypoxic contraction of isolated rat pulmonary artery. *J Pharmacol Exp Ther* 1989; **248**: 952-959
6. IGNARRO LJ, BURKE TM, WOOD KS, WOLIN MS, KADOWITZ PJ: Association between cyclic GMP accumulation and acetylcholine-elicited relaxation of bovine intra pulmonary artery. *J Pharmacol Exp Ther* 1984; **228**: 682-690
7. PALMER RMJ, FERRIGE AG, MONCADA S: Nitric Oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; **327**: 524-526
8. DE MEY JG, VANHOUTTE PM: Heterogeneous behavior of the canine arterial and venous wall. *Circ Res* 1982; **51**: 439-447
9. 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* 1989; **65**: 1508-1515
10. RODMAN DM, YAMAGUCHI T, HASUNUMA K, O'BRIEN RF, MCMURTRY IF: Effects of hypoxia on endothelium-dependent relaxation of rat pulmonary artery. *Am J Physiol* 1990; **258**: 207-214
11. BRASHERS VL, PEACH MJ, ROSE CE: Augmentation of hypoxic pulmonary vasoconstriction in the isolated perfused rat lung by in vitro antagonists of endothelium-dependent relaxation. *J*

- Clin Invest* 1988; **82**: 1495–1502
12. ARCHER SL, TOLINS JP, RAU L, WEIR EK: Hypoxic pulmonary vasoconstriction is enhanced by inhibition of the synthesis of an endothelium-derived relaxing factor. *Biochem Biophys Res Commun* 1989; **164**: 1198–1205
 13. WIKLUND NP, PERSSON MG, GUSTAFSSON LE, MONCADA S, HEDQVIST P: Modulatory role of endogenous nitric oxide in pulmonary circulation in vivo. *Eur J Pharmacol* 1990; **185**: 123–124
 14. PALMER RMJ, MONCADA S: A novel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. *Biochem Biophys Res Commun* 1989; **158**: 348–352
 15. FURCHGOTT RF, ZAWADZKI JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; **288**: 373–376
 16. OHNO M, OCHIAI M, TAGUCHI J, HARA K, AKATSUKA N, KUROKAWA K: Stretch may enhance the release of endothelium-derived relaxing factor in rabbit aorta. *Biochem Biophys Res Commun* 1990; **173**: 1038–1042
 17. SUNANO S, KANEKO K, SAKAKI F, SHIMAMURA K: Effects of L-nitroarginine on alpha-agonists-induced contraction of Wistar Kyoto rats. *Jpn J Pharmacol* 1992; **58**(Supp 2), 316P
 18. HOUSTON DS, VANHOUTTE PM: Comparison of serotonergic receptor subtypes on the smooth muscle and endothelium of the canine coronary artery. *J Pharmacol Exp Ther* 1988; **244**: 1–10
 19. HOSHINO Y, OBARA H, KUSUNOKI M, FUJII Y, IWAI S: Hypoxic contractile response in isolated human pulmonary artery: role of calcium ion. *J Appl Physiol* 1988; **65**(6): 2468–2474
 20. RUBANYI GM, VANHOUTTE PM: Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am J Physiol* 1986; **250**: H822–827
 21. RENGASAMY A, JOHNS RA: Characterization of endothelium-derived relaxing factor/nitric oxide synthase from bovine cerebellum and mechanism of modulation by high and low oxygen tension. *J Pharmacol Exp Ther* 1991; **259**: 310–316
 22. WADSWORTH RM, AMATYC MP, KWAN YW, KANE KA, ZEITLIN IJ: Modulation by hypoxia of the release of endothelium-derived mediators from sheep coronary artery rings; *In*: RUBANYI GM, VANHOUTTE PM, eds. Endothelium-derived contracting factors. Basel: Karger, 1990: 32–38
 23. BIAGGIONI I, KING LS, ENAYAT N, ROBERTSON D, NEWMAN JH: Adenosine produces pulmonary vasoconstriction in sheep. Evidence for thromboxane A₂/prostaglandin endoperoxide-receptor activation. *Circ Res* 1989; **65**: 1516–1525
 24. RUBANYI GM, VANHOUTTE PM: Hypoxia releases a vasoconstrictor substance from the canine vascular endothelium. *J Physiol* 1985; **364**: 45–56
 25. VANHOUTTE PM, KATSUSIC ZS: Endothelium-derived contracting factor: endothelin and/or superoxide anion? *Trends Pharmacol Sci* 1988; **9**: 229–230
 26. YANAGISAWA M, KURIHARA H, KIMURA S, TOMOBE Y, KOBAYASHI M, MITSUI Y, YAZAKI Y, GOTO K, MASAKI T: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988; **332**: 411–415
 27. O'BRIEN RF, ROBBINS RJ, MCMURTRY IF: Endothelial cells in culture produce a vasoconstriction substance. *J Cell Physiol* 1987; **132**: 263–270
 28. VANHOUTTE PM, AUCH-SCHWELK W, BOULANGER PA, JANSSEN PA, KATSUSIC ZS, KOMORI K, MILLER VM, SCHINI VB, VIDAL M: Dose endothelin-1 mediate endothelium-dependent contractions during anoxia? *J Cardiovasc Pharmacol* 1989; **13**(Suppl 5): 124–128
 29. SCHMIDT DJ, KOTH D, JUBENVILLE D, HIGHSMITH RF: Effect of oxygen deprivation and reoxygenation on endothelium-induced contractions of porcine coronary artery; *In*: RUBANYI GM, VANHOUTTE PM, eds. Endothelium-derived contracting factors. Basel: Karger, 1990: 221–231
 30. DAUT J, MAIER-RUDOLPH W, VON BECKERATH N, MEHRKE G, GUNTHER K, GOEDEL-MEINEN L: Hypoxic dilation of coronary arteries is mediated by ATP-sensitive potassium channels. *Science* 1990; **247**: 1341–1344