Original Paper

Journal of Biomedical Science

J Biomed Sci 2002;9:206–212

Received: October 2, 2001
Accepted: November 19, 2001

Chronic Hypoxia Attenuates Nitric Oxide-Dependent Hemodynamic Responses to Acute Hypoxia

Kun-Lun Huang[a,b] Chin-Pyng Wu[a] Bor-Hwang Kang[b] Yu-Chong Lin[c]

[a]Department of Critical Care Medicine, Tri-Service General Hospital, [b]Institute of Undersea and Hyperbaric Medicine, National Defense Medicine Center, Taipei, Taiwan, ROC;
[c]Department of Physiology, University of Hawaii at Manoa, Hawaii, USA

Key Words
Systemic vasodilation · Pulmonary vasoconstriction · Hemodynamics · Nitric oxide

Abstract
Alterations in the nitric oxide (NO) pathway have been implicated in the pathogenesis of chronic hypoxia-induced pulmonary hypertension. Chronic hypoxia can either suppress the NO pathway, causing pulmonary hypertension, or increase NO release in order to counteract elevated pulmonary arterial pressure. We determined the effect of NO synthase inhibitor on hemodynamic responses to acute hypoxia (10% O_2) in anesthetized rats following chronic exposure to hypobaric hypoxia (0.5 atm, air). In rats raised under normoxic conditions, acute hypoxia caused profound systemic hypotension and slight pulmonary hypertension without altering cardiac output. The total systemic vascular resistance (SVR) decreased by 41 ± 5%, whereas the pulmonary vascular resistance (PVR) increased by 25 ± 6% during acute hypoxia. Pretreatment with N^{ω}-nitro-*L*-arginine methyl ester (L-NAME; 25 mg/kg) attenuated systemic vasodilatation and enhanced pulmonary vasoconstriction. In rats with prior exposure to chronic hypobaric hypoxia, the baseline values of mean pulmonary and systemic arterial pressure were significantly higher than those in the normoxic group. Chronic hypoxia caused right ventricular hypertrophy, as evidenced by a greater weight ratio of the right ventricle to the left ventricle and the interventricular septum compared to the normoxic group (46 ± 4 vs. 28 ± 3%). In rats which were previously exposed to chronic hypoxia (half room air for 15 days), acute hypoxia reduced SVR by 14 ± 6% and increased PVR by 17 ± 4%. Pretreatment with L-NAME further inhibited the systemic vasodilatation effect of acute hypoxia, but did not enhance pulmonary vasoconstriction. Our results suggest that the release of NO counteracts pulmonary vasoconstriction but lowers systemic vasodilatation on exposure to acute hypoxia, and these responses are attenuated following adaptation to chronic hypoxia.

Introduction

Hypoxic pulmonary vasoconstriction improves gas exchange in the lungs with localized hypoventilation by maintaining matched ventilation and perfusion. Hypoxia causes pulmonary vasoconstriction either by directly altering oxidative phosphorylation of smooth muscle [28] or by inhibiting the potassium current [23, 30]. Indirectly, hypoxia enhances the release of vasoconstrictors such as endothelin [7, 24, 25]. In contrast, redistribution of pulmonary blood flow in lungs with acute global hypoxia

KARGER
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

S. Karger AG, Basel
1021–7770/02/0093–0206$18.50/0
Accessible online at:
www.karger.com/journals/jbs

Dr. Kun-Lun Huang
National Defense Medical Center, Institute of Undersea and Hyperbaric Medicine
PO Box 90048-516, 161 Ming-Chuan E. Road, Sec. 6
Taipei 114, Taiwan (ROC)
Tel./Fax +886 2 8792 4873, E-Mail kun@ndmctsgh.edu.tw

plays a minimal role in improving the ventilation-perfusion mismatch. In theory, vasodilator(s) should be released during global hypoxia to counteract the inherent constrictive response of the pulmonary vasculature and to prevent generalized vasoconstriction. Nitric oxide (NO) has been suggested to be the major vasodilator involved in the regulation of pulmonary vascular resistance (PVR) during acute hypoxia [4].

Chronic alveolar hypoxia, occurring either in chronic mountain disease or sleep apnea syndrome, causes pulmonary hypertension that involves vascular remodeling in prolonged hypoxia [17, 21, 29]. The closure of K^+ channels in precapillary smooth muscle cells causes vasoconstriction which increases shear stress, resulting in vascular remodeling [24, 32]. Some vasoconstrictors are also known to act as growth factors to modulate remodeling of vascular smooth muscle cells [3, 11]. Although alterations in the endothelium-derived NO pathway have been shown in the pulmonary circulation after chronic hypoxia [6, 13], the importance of the NO pathway is unclear. Theoretically, chronic hypoxia could either suppress the NO pathway, causing pulmonary hypertension, or increase NO release in order to counteract elevated pulmonary arterial pressure (PAP).

The purpose of the present study was to assess the role of NO in hypoxia-induced pulmonary hypertension before and after adaptation to chronic hypoxia. We also wanted to test the hypothesis that NO is released during acute hypoxia and minimizes pulmonary hypertension and that chronic hypoxia suppresses both NO synthesis and release, resulting in pulmonary hypertension.

Materials and Methods

Exposure to Chronic Hypoxia

In conducting the experiment, we followed the 'Guiding Principles in the Care and Use of Animals' approved by the Institutional Animal Care and Use Committee. Male Sprague-Dawley rats, weighing 200–220 g, were placed in a hypobaric chamber and the pressure was maintained at 0.5 atm by a vacuum pump. The CO_2 inside the chamber was removed by a ventilation flow rate of 20 liters/min and by passing the gas over silica gel. The exposure was continuous except for a 30-min period every other day during which the animals were fed and the chambers were cleaned. The animals remained in the chamber for either 15 days (CH-15 group) or 30 days (CH-30 group) to produce chronic hypoxia. This protocol caused zero mortality. A control age-matched group was housed in room air but otherwise treated in an identical fashion.

Animal Preparations

Each rat was anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) immediately after removal from the hypoxia chamber. The trachea was cannulated with a short piece of polyethylene tubing (PE-240) and connected to a T-shaped adaptor through which the animal inhaled either a normal or hypoxic (10% O_2) gas mixture. The femoral artery and vein were catheterized with PE-50 tubing in order to monitor systemic blood pressure (SAP) and to administer drugs. Pulmonary artery catheterization was performed as described by Hayes and Will [10] and the PAP was continuously monitored. In brief, a 25-gauge (outside diameter 0.92 mm, inside diameter 0.45 mm) Teflon catheter was heated and curved to an 'R' shape. The tubing was cooled in ice water and trimmed to the desired length (4–5 mm from the tip to the top of the catheter arch). We modified this method by adding a piece of PE-10 tubing (3–4 mm in length) to the tip of the Teflon catheter. The tip of the PE-10 tubing was blunted by solder heating in order to lessen trauma during catheterization. A PE-160 cannula (5 cm in length) with a 15° bend at its tip served as a sleeve for the catheter. The sleeve straightened the Teflon catheter temporarily during catheterization. The catheter was filled with heparinized saline and connected to a pressure transducer before it was inserted into the jugular vein and advanced into the right ventricle. Once the right ventricular pressure waveform registered, the catheter was gently pushed forward while keeping the sleeve stationary. As the loop of the catheter entered the right ventricle, the sleeve was withdrawn while keeping the catheter in place. The catheter was repositioned slightly and secured to the neck muscles by sutures when the PAP recording was satisfactory.

Measurement of Cardiac Output

The cardiac output was measured using a Cardiomax II thermodilution system (Columbus Inc., Columbus, Ohio, USA). Room temperature saline (0.4 ml) was injected into the pulmonary artery by an autoinjector and the blood temperature was measured using a thermistor placed in the ascending aorta. A thermal probe was immersed in the injection reservoir in order to monitor the temperature of the saline. Cardiomax II calculated the cardiac output based on the resulting thermodilution curve. The systemic vascular resistance (SVR) and PVR were calculated by dividing the mean SAP (MSAP) and mean PAP (MPAP) by cardiac output.

Nitrite/Nitrate Determination

The nitrite/nitrate (NOx^-) in the plasma was determined using a Sievers-280 NO analyzer (Sievers Inc., Boulder, Colo., USA). The plasma was deproteinized with an equal volume of ice-cold absolute ethanol. The NOx in the supernatant was then reduced with vanadium to NO, which in turn reacts with O_3 to produce $\cdot NO_2$ in an excited state. The energy emitted from $\cdot NO_2$ was then detected by chemiluminescence, and the concentration of NOx^- was measured using a standard curve composed of a range of sodium nitrite concentrations.

Experimental Protocols

Within 10 min after intubation and catheterization, 0.3 ml of blood was collected from the femoral artery catheter into an ice-chilled centrifuge tube for blood gas analysis (IL1610, Instrumentation Laboratory, Milan, Italy), and NOx^- and baseline cardiac output were measured before exposure to acute hypoxia. The inhaled gas was switched from normal air to an hypoxic gas mixture (10% O_2 balanced with N_2) for 5 min. Before ending the hypoxic exposure period, another blood sample was collected and cardiac output was again measured. After the hemodynamic changes returned to baseline, N^{ω}-nitro-*L*-arginine methyl ester (L-NAME; Sigma, St. Louis,

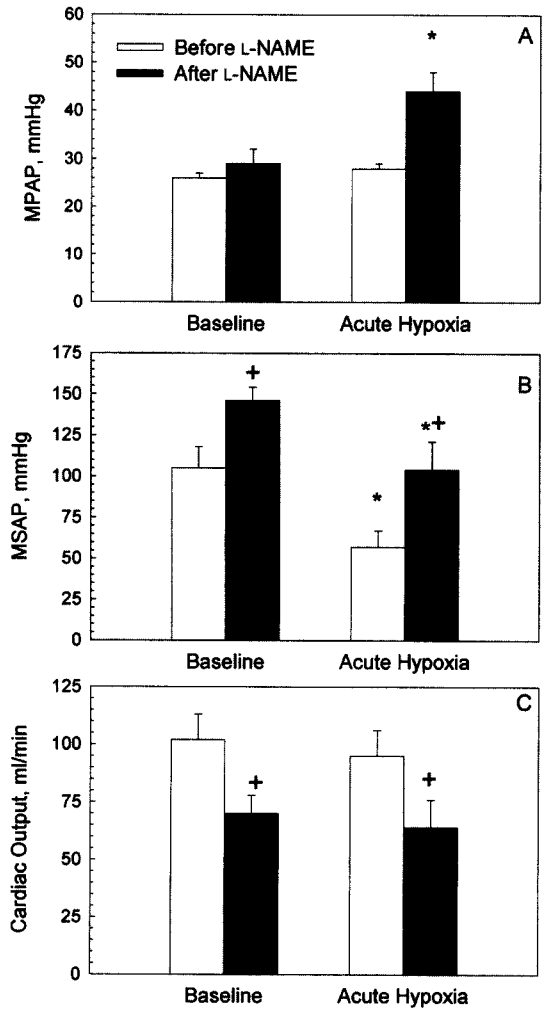

Fig. 1. Hemodynamic responses to acute hypoxic challenge before and after L-NAME pretreatment in control rats. * $p < 0.05$ compared to baseline values; $^{+}$ $p < 0.05$ compared to values before L-NAME pretreatment.

Mo., USA) was injected into the femoral vein catheter at a dosage of 25 mg/kg. Ten minutes following the L-NAME injection, cardiac output measurements and hypoxic exposures were repeated. The animal was then sacrificed using an overdose of sodium pentobarbital. The heart was excised and the weights of the right ventricle (RV), left ventricle (LV) and interventricular septum (S) were determined to calculate the change in the ratio of right ventricular weight to the sum of the left ventricular and septal weights [RV/(LV + S)].

All data were expressed as mean ± SD. The differences between parameters measured before and during acute hypoxia, and those measured before and after L-NAME treatment, and the statistical

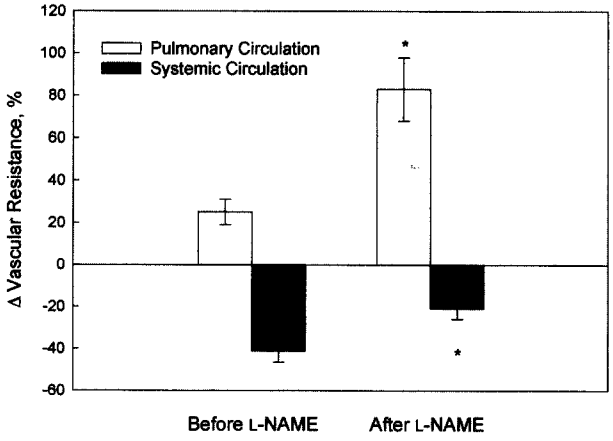

Fig. 2. Percentage change in vascular resistance in the pulmonary and systemic circulations during acute hypoxic challenge in the control rats before and after L-NAME pretreatment. * $p < 0.05$ compared to values before L-NAME pretreatment.

significance between groups were evaluated using two-way ANOVA with repeated measurements. A value of $p < 0.05$ was accepted as statistically significant.

Results

Hemodynamic Responses to Acute Hypoxia

Exposure to 10% O_2 reduced the PaO_2 from 92 ± 2 to 34 ± 2 mm Hg and the $PaCO_2$ from 45 ± 2 to 39 ± 3 mm Hg in 5 min (n = 12). In the normoxic group (fig. 1A), acute hypoxia did not elevate PAP, but did elicit a significant hypotensive response in the systemic circulation (fig. 1B). The cardiac output during acute hypoxia was not different from the baseline level (fig. 1C). Intravenous injection of L-NAME caused a significant increase in SAP (fig. 1B) and a decrease in cardiac output (fig. 1C), but no significant increase in PAP occurred. After L-NAME pretreatment, acute hypoxia induced a less hypotensive response in SAP (fig. 1B) but a significant increase in PAP (fig. 1A).

Figure 2 summarizes the changes measured in vascular resistance during exposure to acute hypoxia. Acute hypoxia increased PVR and decreased SVR. L-NAME significantly increased PVR by 53 ± 12% and SVR by 105 ± 12% (table 1). L-NAME pretreatment significantly enhanced the increase in PVR during acute hypoxia and attenuated the decrease in SVR.

Pulmonary Hypertension and Right Ventricle Hypertrophy

Exposure to hypobaric hypoxia for 15 (CH-15) or 30 (CH-30) days induced significant elevations in MPAP, MSAP, PVR, SVR and RV/LV + S compared to values in control rats raised in normoxic air (table 1). The heart rate was slightly lower in the CH-15 group, and the decrease became statistically significant after exposure to hypoxia for 30 days. The baseline cardiac output was not altered after chronic hypoxia either for 15 or 30 days. The MPAP, MSAP, PVR, SVR and RV/LV + S in the CH-15 group were not statistically different from those in the CH-30 group.

In the chronic hypoxic rats, L-NAME significantly increased the MSAP and the SVR, but did not cause an additional increase in the MPAP. However, since a significant decrease occurred in cardiac output after L-NAME, the PVR actually increased by 54 ± 19 and 65 ± 10% in the CH-15 group and CH-30 group, respectively (table 1). Also, the difference between the CH-15 and CH-30 groups was not significant for any measure.

Effects of Chronic Hypoxia on Hemodynamic Responses to Acute Hypoxia

In the CH-15 group before L-NAME treatment, acute hypoxia elicited a slight, but significant decrease in MSAP (fig. 3), and this systemic hypotensive effect was inhibited

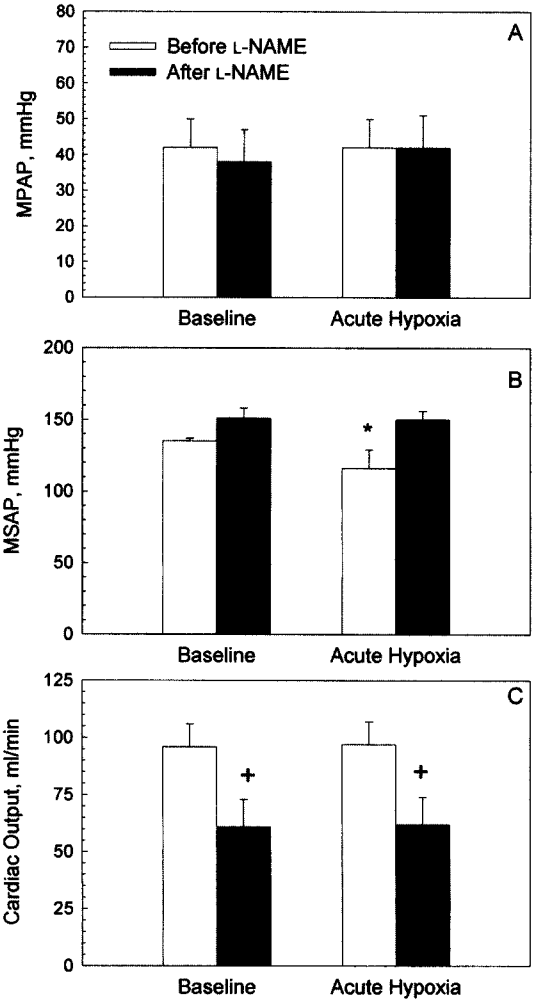

Fig. 3. Hemodynamic responses to acute hypoxic challenge before and after L-NAME pretreatment in rats with prior exposure to chronic hypoxia for 15 days (CH-15). * $p < 0.05$ compared to baseline values; $^{+}$ $p < 0.05$ compared to values before L-NAME pretreatment.

Table 1. Effects of chronic hypoxic exposure and L-name on the baseline hemodynamics in rats

Group	Number		MSAP mm Hg	MPAP mm Hg	HR bpm	CO ml/min	SVR mm Hg/ml/min	PVR mm Hg/ml/min	RV/LV + S %
Control	12	before L-NAME	99 ± 5	26 ± 3	392 ± 43	102 ± 11	1.03 ± 0.09	0.20 ± 0.01	28 ± 3
		after L-NAME	146 ± 8*	28 ± 2	343 ± 51*	70 ± 8*	2.11 ± 0.24*	0.30 ± 0.05*	
CH-15	12	before L-NAME	135 ± 2^{+}	42 ± 8^{+}	358 ± 40	96 ± 10	1.42 ± 0.15^{+}	0.44 ± 0.08^{+}	46 ± 4^{+}
		after L-NAME	151 ± 7*	38 ± 9	341 ± 63*	61 ± 12*	2.66 ± 0.54*	0.67 ± 0.19*,$^{+}$	
CH-30	6	before L-NAME	143 ± 10^{+}	43 ± 3^{+}	332 ± 49^{+}	103 ± 15	1.14 ± 0.23^{+}	0.42 ± 0.05^{+}	45 ± 7^{+}
		after L-NAME	152 ± 7*	41 ± 4	292 ± 51*	61 ± 16*	2.61 ± 0.61*	0.70 ± 0.16*,$^{+}$	

Data are expressed as mean ± SD. * $p < 0.05$ compared to values before L-NAME pretreatment; $^{+}$ $p < 0.05$ compared to the control group.
HR = Heart rate; CO = cardiac output.

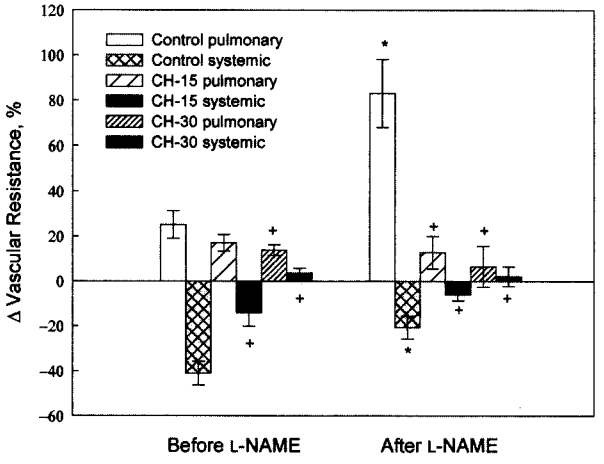

Fig. 4. Percentage change in vascular resistance in the pulmonary and systemic circulations during acute hypoxic challenge in chronic hypoxic rats before and after L-NAME pretreatment.* $p < 0.05$ compared to values before L-NAME pretreatment; $^{+}$ $p < 0.05$ compared to values in the control rats.

by L-NAME. The MPAP was not altered by acute hypoxia before or after L-NAME treatment in the chronic hypoxic rats. There was no difference between the cardiac output at baseline and that during acute hypoxia (fig. 3C), but L-NAME decreased cardiac output, which was not affected by acute hypoxia. Hemodynamic responses to acute hypoxia in the CH-30 group were similar to those in the CH-15 group.

Figure 4 summarizes the effects of chronic hypoxia on changes in PVR and SVR during exposure to acute hypoxia. Compared to the normoxic group, chronic exposure to hypoxia for 15 days significantly attenuated the systemic vasodilatation induced by acute hypoxic challenge. Prior exposure to hypoxia for 30 days attenuated the pulmonary vasoconstriction and totally abolished the systemic vasodilatation induced by acute hypoxia. Exposure to chronic hypoxia for 15 or 30 days abolished the L-NAME-enhanced hypoxic pulmonary vasoconstriction compared to the normoxic group.

Discussion

Hypoxic pulmonary vasoconstriction during alveolar hypoxia has been well documented in the literature [28, 30]. Vasoconstriction of the hypoxic areas in the lung plays an important role in shunting blood flow away from these areas in order to maintain a better matched ventilation/perfusion ratio. This redistribution of pulmonary blood flow improves arterial oxygenation. In lungs with global hypoxia, a redistribution of the pulmonary blood flow cannot be effective in improving blood oxygenation. Therefore, generalized pulmonary vasoconstriction and pulmonary hypertension are unlikely to occur during acute global hypoxia. However, hypoxic vasoconstriction has been demonstrated in pulmonary artery rings [14, 32] and in single pulmonary artery smooth muscle cells [16]. These data imply that pulmonary artery smooth muscle cells contract in hypoxic lung areas and also during generalized hypoxia. This vasoconstriction could be counteracted by the release of vasodilators in vivo, since hypoxic exposure usually induces a transient initial increase in PAP followed by a gradual pressure decline to baseline levels [12, 26, 27]. In our study, acute hypoxia for 5 min did not alter the PAP, which is similar to other studies which showed unaltered PAP during acute exposure to hypoxia [4, 12].

Acute hypoxia did cause pronounced systemic hypotension, which resulted from significant vasodilatation, since cardiac output did not change (fig. 1C). Hypoxia reduces the availability of oxygen, and local blood flow increases to maintain a constant supply of oxygen. However, as hypotension develops, the local blood flow decreases and the oxygen supply worsens. Our results suggest that NO causes hypotension to occur during acute hypoxia. Administration of L-NAME elevated systemic blood pressure and also depressed the cardiac output, indicating that NO is responsible for the regulation of basal vascular tone. In acute hypoxia, pretreatment with L-NAME attenuated the systemic hypotension without depressing the cardiac output, indicating that NO causes systemic vasodilatation induced by acute hypoxic challenge. Furthermore, the dominance of NO in the control of regional blood flow has been well documented [8, 22] in models of autoregulatory vasodilation. Therefore, hypoxic vasodilatation may be a consequence of a synergistic effect of NO with other local vasodilators.

Although NO was demonstrated pharmacologically in these studies, no direct NO measurements were done. Interestingly, we failed to detect a significant increase in NOx^- in the plasma during a hypoxic challenge. This may have been due to measurement sensitivity or the release of NO in bursts, which could be difficult to detect by a discrete sampling technique. Pharmacological inhibition of NO synthesis [19, 20] or its pathways [18] has been widely used to identify the role of NO production in a variety of

conditions, and this method was also used in the present study. Bioassays of endothelium-derived relaxing factor are also used to evaluate NO production [9]. The concentration of NO and its metabolites certainly attains detectable levels in the serum when a specific challenge such as endotoxin [15] is studied over time. Although we did not demonstrate a change in the serum level of NO, our results clearly showed that inhibition of NO synthesis by L-NAME attenuated the hypoxic systemic vasodilatation, which suggests NO involvement.

The NO release during acute hypoxia could be responsible for maintaining a stable PAP even when pulmonary vasoconstriction has occurred. Pulmonary vasoconstriction induced by a thromboxane analogue U46619 increases pulmonary NO synthesis and circulating cGMP [31]. Our results showed that acute hypoxia alone did not alter the PAP. However, pretreatment with L-NAME caused a significant increase in pulmonary resistance and the resulting PAP during hypoxic challenge (fig. 1A), which suggests that NO is responsible for maintaining low pulmonary resistance in low-oxygen environments. It is interesting that the baroreflex is not activated during acute hypoxia to accelerate the heart rate or to increase cardiac output in order to compensate for systemic vasodilatation. It has not been reported that stable pulmonary pressure is more important than maintaining a constant systemic blood pressure. However, our results certainly suggest that during acute hypoxia, pulmonary homeostasis somehow supersedes the baroreflex, which should have been elicited by profound hypotension. The mechanism by which the baroreflex is overridden during acute hypoxia is presently not known.

Chronic hypoxia causes both pulmonary and systemic hypertension. The consequence is cardiac hypertrophy, particularly in the right ventricle as shown in table 1. The hypertension is due to increased vascular resistance and vascular remodeling [17, 21, 29]. Although a decrease in the release or synthesis of vasodilators could also result in hypertension, the relation between NO and pulmonary hypertension after chronic hypoxia is not clearly defined. Evidence shows that chronic hypoxia decreases NO production and NO synthase (NOS) activity [1, 5, 6]. Also, hypoxia-induced pulmonary hypertension causes multiple disruptions in the NO pathway, including the cGMP-dependent mechanism [2]. These findings suggested that chronic hypoxia reduces NOS activity, contributing to the pathogenesis of pulmonary hypertension. In contrast, an increased NO concentration was found in the effluent from isolated lungs of chronically hypoxic rats [13]. The mRNA expression and protein synthesis of inducible NOS showed significant increases in lungs from rats with hypoxic pulmonary hypertension [12]. Our results clearly showed that injection of L-NAME alone in rats raised in a normoxic environment or after chronic hypoxia caused a similar degree of vasoconstriction and a reflex lowering of cardiac output (table 1), indicating that exposure to chronic hypoxia did not reduce NOS activity.

Our results also showed that chronic hypoxia abolishes the vasodilatation response to acute hypoxia in the pulmonary circulation and attenuates the response in the systemic circulation (fig. 4). Chronic hypoxia may inhibit the burst of NO release occurring during acute hypoxia and may blunt activation of the NO pathway. Since we did not detect a change in plasma NOx^- during hypoxic challenge for 5 min, it is not possible to distinguish between either a reduced capability of NO release or a depressed vascular sensitivity to NO after prolonged hypoxic exposure. Nevertheless, our results showed that prolonged hypoxic exposure in rats depresses the NO-dependent cardiovascular responses to acute hypoxia.

In summary, our study in anesthetized rats showed that acute hypoxia caused pronounced systemic hypotension without any significant elevation of PAP. NO mediates the systemic hypotension and plays an important role in opposing the pulmonary vasoconstriction associated with acute exposure to hypoxia. Prolonged hypoxic exposure results in persistent hypertension, both in the systemic and pulmonary circulations, and hypertrophy of the right ventricle. The NO-dependent vasodilatation associated with acute hypoxia vanished following prolonged exposure to hypoxia. In conclusion, chronic hypoxia attenuates NO-dependent systemic vasodilatation in response to acute hypoxia and minimizes the role of NO in maintaining pulmonary homeostasis.

Acknowledgments

We wish to thank Dr. Aubrey E. Taylor for advice and helpful comments. This study was supported by Grant NSC-89-2314-B-016-036 from the National Science Council, ROC.

References

1 Adnot S, Raffestin B, Braquet S, Chabrier PE. Loss of endothelium-dependent relaxant activity in the pulmonary circulation of rats exposed to chronic hypoxia. J Clin Invest 87:155–162; 1991.

2 Berkenbosch JW, Baribeau J, Perreault T. Decreased synthesis and vasodilation to nitric oxide in piglets with hypoxia-induced pulmonary hypertension. Am J Physiol 278:L276–L283; 2000.

3 Bialecki RA, Fisher CS, Murdoch WW, Barthlow HG, Stow RB, Mallamaci M, Rumsey W. Hypoxic exposure time dependently modulates endothelin-induced contraction of pulmonary artery smooth muscle. Am J Physiol 274:L552–L559;1998.

4 Blitzer ML, Loh E, Roddy MA, Stamler JS, Creager MA. Endothelium-derived nitric oxide regulates systemic and pulmonary vascular resistance during acute hypoxia in humans. J Am Coll Cardiol 28:591–596;1996.

5 Carville C, Raffestin B, Eddahibi S, Blouquit Y, Adnot S. Loss of endothelium-dependent relaxation in proximal pulmonary arteries from rats exposed to chronic hypoxia: Effects of in vivo and in vitro supplementation with L-arginine. J Cardiovasc Pharmacol 22:889–896;1993.

6 Fike CD, Kaplowitz MR, Thomas CJ, Nelin LD. Chronic hypoxia decreases nitric oxide production and endothelial nitric oxide synthase in newborn pig lungs. Am J Physiol 274: L517–L526;1998.

7 Franco-Cereceda A, Holm P. Selective or nonselective endothelin antagonists in porcine hypoxic pulmonary hypertension? J Cardiovasc Pharmacol 31(suppl 1):S447–S452;1998.

8 Gardiner SM, Compton AM, Bennett T, Palmer RM, Moncada S. Control of regional blood flow by endothelium-derived nitric oxide. Hypertension 15:486–492;1990.

9 Gryglewski RJ, Moncada S, Palmer RM. Bioassay of prostacyclin and endothelium-derived relaxing factor (EDRF) from porcine aortic endothelial cells. Br J Pharmacol 87:685–694;1986.

10 Hayes BE, Will JA. Pulmonary artery catheterization in the rat. Am J Physiol 235:H452–H454;1978.

11 Holm P. Endothelin in the pulmonary circulation with special reference to hypoxic pulmonary vasoconstriction. Scand Cardiovasc J Suppl 46:1–40;1997.

12 Igari H, Tatsumi K, Sugito K, Kasahara Y, Saito M, Tani T, Kimura H, Kuriyama T. Role of EDRF in pulmonary circulation during sustained hypoxia. J Cardiovasc Pharmacol 31: 299–305;1998.

13 Isaacson TC, Hampl V, Weir EK, Nelson DP, Archer SL. Increased endothelium-derived NO in hypertensive pulmonary circulation of chronically hypoxic rats. J Appl Physiol 76: 933–940;1994.

14 Jin N, Packer C, Rhoades R. Pulmonary arterial hypoxic contraction: Signal transduction. Am J Physiol 263:L73–L78;1992.

15 Kristof AS, Goldberg P, Laubach V, Hussain SN. Role of inducible nitric oxide synthase in endotoxin-induced acute lung injury. Am J Respir Crit Care Med 158:1883–1889;1998.

16 Madden J, Vadula M, Kurup V. Effects of hypoxia and other vasoactive agents on pulmonary and cerebral artery smooth muscle cells. Am J Physiol 263:L384–L393;1992.

17 Mecham RP, Whitehouse LA, Wrenn DS, Parks WC, Griffin GL, Senior RM, Crouch EC, Stenmark KR, Voelkel NF. Smooth muscle-mediated connective tissue remodeling in pulmonary hypertension. Science 237:423–426; 1987.

18 Moncada S, Palmer RM, Gryglewski RJ. Mechanism of action of some inhibitors of endothelium-derived relaxing factor. Proc Natl Acad Sci USA 83:9164–9168;1986.

19 Moncada S, Rees DD, Schulz R, Palmer RM. Development and mechanism of a specific supersensitivity to nitrovasodilators after inhibition of vascular nitric oxide synthesis in vivo. Proc Natl Acad Sci USA 88:2166–2170;1991.

20 Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327:524–526;1987.

21 Rabinovitch M, Gamble W, Nadas AS, Miettinen OS, Reid L. Rat pulmonary circulation after chronic hypoxia: Hemodynamic and structural features. Am J Physiol 236:H818–H827;1979.

22 Rees DD, Palmer RM, Moncada S. Role of endothelium-derived nitric oxide in the regulation of blood pressure. Proc Natl Acad Sci USA 86:3375–3378;1989.

23 Reeve HL, Weir EK, Nelson DP, Peterson DA, Archer SL. Opposing effects of oxidants and antioxidants on K^+ channel activity and tone in rat vascular tissue. Exp Physiol 80:825–834; 1995.

24 Sato K, Morio Y, Morris KG, Rodman DM, McMurtry IF. Mechanism of hypoxic pulmonary vasoconstriction involves ET_A receptor-mediated inhibition of K_{ATP} channel. Am J Physiol 278:L434–L442;2000.

25 Shirakami G, Nakao K, Saito Y, Magaribuchi T, Jougasaki M, Mukoyama M, Arai H, Hosoda K, Suga S, Ogawa Y. Acute pulmonary alveolar hypoxia increases lung and plasma endothelin-1 levels in conscious rats. Life Sci 48: 969–976;1991.

26 Sylvester JT, Harabin AL, Peake MD, Frank RS. Vasodilator and constrictor responses to hypoxia in isolated pig lungs. J Appl Physiol 49:820–825;1980.

27 Tucker A, Reeves JN. Nonsustained pulmonary vasoconstriction during acute hypoxia in anesthetized dogs. Am J Physiol 228:756–761; 1975.

28 Voelkel NF. Mechanism of hypoxic pulmonary vasoconstriction. Am Rev Respir Dis 133: 1186–1195;1986.

29 Voelkel NF, Tuder RM. Hypoxia-induced pulmonary vascular remodeling: A model for what human disease? J Clin Invest 106:733–738; 2000.

30 Weir EK, Archer SL. The mechanism of acute hypoxic pulmonary vasoconstriction: The tale of two channels. FASEB J 9:183–189;1995.

31 Wilson PS, Thompson WJ, Moore TM, Khimenko PL, Taylor AE. Vasoconstriction increases pulmonary nitric oxide synthesis and circulating cyclic GMP. J Surg Res 70:75–83; 1997.

32 Yuan XJ, Tod M, Rubin L, Blaustein M. Contracting effects of hypoxia on tension in rat pulmonary and mesenteric arteries. Am J Physiol 259:H281–H289;1990.