Hypoxia-induced pulmonary hypertension: Different impact of iloprost, sildenafil, and nitric oxide

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Summary
Objectives: Chronic alveolar hypoxia induces pulmonary hypertension, evident from elevated pulmonary artery pressure (PAP), pulmonary vascular resistance, right ventricular hypertrophy (RVH), and increased muscularization of the pulmonary vasculature. Additionally, the vasoconstrictor response to acute hypoxia (HPV) may be reduced in the remodeled vasculature. However, no direct comparison of different treatments on the various parameters characterizing pulmonary hypertension has been performed yet. Against this background, we compared the effects of inhaled NO, infused iloprost, a stable prostacyclin analogue, and oral sildenafil, a phosphodiesterase 5 inhibitor, on hypoxia-induced pulmonary hypertension.

Methods: Exposure of rabbits to chronic hypoxia (FI O2 = 0.10) for 42 days. Treatment with infused iloprost, oral sildenafil, and inhaled nitric oxide.

Results: We quantified PAP, pulmonary vascular resistance, RVH, vascular remodeling, vasoreactivity, and the strength of HPV. Chronic hypoxia resulted in an increase in (a) the right ventricle/(left ventricle+septum) ratio from 0.26±0.01 to 0.44±0.01, (b) PAP, and (c) the degree of muscularization from 14.0±4.0% to 43.5±5.3%. Treatment with iloprost and sildenafil, but not with NO, prevented the increase in muscularization. In contrast, RVH was strongly inhibited by sildenafil, whereas NO had some minor, and iloprost had no effect. Only iloprost reduced PAP compared to NO and sildenafil. The downregulation of HPV was abrogated only by NO.

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Conclusion: We demonstrated (a) that the parameters characterizing hypoxia-induced pulmonary hypertension are not functionally linked, (b) that the downregulation of HPV under chronic hypoxia can be prevented by inhaled NO but not by sildenafil and iloprost, and (c) that iloprost is particularly effective in preventing vascular remodeling and sildenafil in preventing RVH.

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Introduction

Hypoxia-induced pulmonary hypertension is characterized by increased pulmonary vascular resistance, narrowing of pulmonary vascular lumen due to thickening of the vessel media, changes in functional parameters of the lung vasculature, and right ventricular hypertrophy.1–3 In contrast to these pathophysiological effects of chronic alveolar hypoxia, acute alveolar hypoxia induces a vasoconstrictor response that matches lung perfusion to regional alveolar oxygen delivery, and thereby optimizes pulmonary gas exchange. This process of hypoxic pulmonary vasoconstriction (HPV), however, is also thought to contribute to the development of pulmonary hypertension under conditions of generalized chronic hypoxia. In this context, we recently demonstrated that acute HPV is downregulated after chronic alveolar hypoxia and can be restored by continuous treatment with inhaled nitric oxide.4

Although the underlying mechanisms of hypoxia-induced pulmonary hypertension have not been fully elucidated, it has been shown that vasodilator therapies can reduce the increase in pulmonary vascular resistance, right ventricular hypertrophy, and the increase in the degree of muscularization of the pulmonary vasculature occurring during chronic hypoxia.5–11 However, it is not determined yet, whether the increase in pulmonary vascular resistance, the vascular remodeling process, changes in the strength of HPV, and right ventricular hypertrophy are functionally linked or differentially affected by vasodilators. Therefore, we compared the effects of inhaled NO, oral sildenafil, and intravenous iloprost on these parameters in a rabbit model of hypoxia-induced pulmonary hypertension. Interestingly, different efficacy profiles were noted for the different vasoactive agents, suggesting non-uniformity of action on key variables of pulmonary hypertension.

Materials and methods

Reagents

Krebs-Henseleit-buffer was from Serag-Wiessner (Naila, Germany). U46619 was from Paesel+Lorei (Frankfurt/M, Germany) and Nω-monomethyl-L-arginine was from Sigma (Deisenhofen, Germany). Sildenafil was from Pfizer (Sandwich, UK). Iloprost was from Schering (Berlin, Deutschland). All other biochemicals were obtained from Merck (Munich, Germany). Gases were from Messer-Griesheim (Siegen, Germany).

Chronic hypoxia

All animal experiments were approved by the local ethics committee [Regierungspräsidium Giessen, numbers 17a-10c 20-15(1)-Gi 20/10-3/95 and 25.3-19c 20/15(1)-Gi20/10-20/99]. Rabbits (Chinchilla bastard) of either sex (2.5–3.2 kg) were exposed to normobaric hypoxia [inspiratory O2 fraction (FiO2) 0.10] in a ventilated chamber for 6 weeks. The level of hypoxia was held constant by an autoregulatory control unit (O2 controller model 4010, Labotect, Göttingen, Germany), supplying either nitrogen or oxygen. Excess humidity in the recirculating system was prevented by condensation in a cooling system. CO2 was continuously removed by soda lime. Cages were opened once per day for cleaning as well as food and water supply or refilling of the infusor.

Treatment with NO, iloprost and sildenafil

Animals were treated with inhaled NO, oral sildenafil or intravenously infused iloprost (n = 8 each). The normoxic as well as the hypoxic control group consisted of n = 12 animals each. After 42 days of normoxia or hypoxia, the animals were subjected to the following protocol: quantification of right ventricular pressure in anesthesia (ketamin/xylazine), isolated lung perfusion, and subsequent fixation for histological investigation as well as determination of right ventricular hypertrophy. Due to technical reasons, perfusion as well as the histological investigation was performed for only n = 5 lungs in the iloprost treated animals. Animals were randomly allocated to the different groups.

For treatment with inhaled NO, this agent was continuously flushed into the animal cages to achieve a concentration of 15 ppm. The NO concentration was controlled twice daily by an NO analyzer (Sievers 280 NOA, Sievers Instruments, Boulder, CO, USA). The dosage applied was selected from effective therapeutic and pulmonary vasodilatory dosages previously determined in rabbits in our laboratory as well as in humans. These dosages ranged approximately 10–200 ppm.12–14 To avoid significant NO2 formation (which was continuously controlled in the hypoxic cages by a Dräger Multiwarn II [Dräger, Lübeck, Germany]) and protein nitration15 the highest tolerable dosage with regard to NO2 insufflation into the hypoxia cages was determined at 15 ppm which was then used for treatment in the present study.

For treatment with sildenafil, this agent was added to the drinking water. Rabbits received 4.3 mg/kg body weight/day. The dosage was selected upon our previous finding and as high as possible without significant systemic
Treatment of hypoxia-induced pulmonary hypertension

vasodilation. Drinking volume was measured daily over a period of 1 week before onset of treatment to calculate the uptake of water per day. The concentration of sildenafil in the drinking water was then adjusted accordingly. During the 6-week period of treatment, no significant alterations in the daily water consumption were observed.

For treatment with iloprost, this agent was infused continuously via a Hickman catheter into the left jugular vein in a dose of 15 mg/kg body weight/min by an infusion pump (Multidayinfusor 0.5 ml/h, Baxter, Lessins, Belgium). The dosage was determined in previous investigations from our laboratory to result in significant pulmonary vascular vasodilation without a decrease in systemic vascular resistance. The infusion catheter was tunneled through the neck and connected to the infusor on the back of the animal. Implantation of the catheter was performed under anesthesia by intravenous application of ketamine (15 mg/kg body weight) and xylazine (3–5 mg/kg body weight) 7 days before onset of treatment of the animals. Until onset of treatment, all animals received 0.2 ml/kg body weight/day Baytril 10% (Bayer, Leverkusen, Germany) and 250 U heparin/kg body weight/day. The animals of the hypoxic and normoxic control group received a continuous infusion of saline.

Treatment was started in parallel with the onset of hypoxia. Rabbits exposed to normobaric normoxia were kept in a similar chamber as the hypoxic animals but at an FIO2 of 0.21.

After 42 days of exposure to either chronic hypoxia or normoxia, right ventricular pressure was measured by means of a pulmonary arterial catheter in ventilated (30 strokes/min, tidal volume 30 ml, room air) and anaesthetized animals [combined intravenous application of ketamine (30–50 mg/kg body weight) and xylazine (6–10 mg/kg body weight); anticoagulation was achieved by intravenous application of heparin (1000 U/kg body weight)]. For mechanical ventilation, an endotracheal tube was introduced into the trachea and the animals were ventilated with room air. After measurement of pulmonary artery pressure, lungs were investigated in an isolated lung preparation and hearts were removed to calculate right ventricular wall (RV) to left ventricular wall (LV) plus septum ratio of the dried heart tissue.

Lung isolation, perfusion and ventilation

The model of isolated perfused rabbit lungs has been described previously. Briefly, rabbits were deeply anaesthetized as stated above and ventilated with room air. The lungs were excised while being perfused with Krebs Henseleit buffer through cannulas in the pulmonary artery and the left atrium. The buffer contained 125.0 mM NaCl, 4.3 mM KCl, 1.1 mM KH2PO4, 2.4 mM CaCl2, 1.3 mM MgCl2 and 275 mg glucose per 100 ml; NaHCO3 was adjusted to result in a constant pH range of 7.37–7.40. After rinsing the lungs with at least 11 of buffer fluid for washout of blood, the perfusion circuit was closed for recirculation (total system volume 250 ml). Meanwhile, the flow was slowly increased from 20 to 150 ml/min, and left atrial pressure was set at 1.5–2.0 mmHg to ensure zone III conditions throughout the lung at end expiration. The alternate use of two separate perfusion circuits allowed repeated exchange of buffer fluid. In parallel with the onset of artificial perfusion, ventilation was changed from room air to a mixture of 5.3% CO2, 21.0% O2, balanced by N2 (tidal volume, 30 ml; frequency, 30 strokes/min). A positive end-expiratory pressure (PEEP) of 1 cm H2O was chosen (0 referenced at the hilum). The isolated perfused lungs were placed in a temperature-equilibrated housing chamber, freely suspended from a force transducer for continuous monitoring of organ weight. The whole system (perfusate reservoirs, tubing, housing chamber) was heated to 38.5 °C. Pressures in the pulmonary artery, the left atrium and the trachea were registered by means of small-diameter tubing threaded into the perfusion catheters and the trachea and connected to pressure transducers. Lungs included in the study were those that (i) had a homogeneous white appearance with no signs of hemostasis, edema or atelectasis, (ii) revealed constant mean pulmonary artery and peak ventilation pressure in the normal range, and (iii) were isogravimetric during the initial steady state period of at least 20 min. Weight increase ranged <3 g throughout the entire experiments.

Hypoxic maneuvers and pharmacological challenges

The technique of successive hypoxic maneuvers in buffer-perfused rabbit lungs has been described previously. Briefly, a gas-mixing chamber (KM 60-3/GMERS, WITT, Witten, Germany) was employed for step-changes in the ventilator O2 content [21% (v/v) (alveolar PO2 ~160 mmHg, baseline conditions) to 3% (v/v) (alveolar PO2 ~23 mmHg, hypoxic conditions)]. 5.3% (v/v) CO2 was used throughout, and the percentage of N2 was balanced accordingly. Two hypoxic maneuvers of 10-min duration, interrupted by a 15-min period of normoxia, were performed. Fifteen minutes after cessation of hypoxia two bolus applications of the stable thromboxane mimetic U46619 (addition at an interval of 15 min to the perfusate at 0.5 nM) were undertaken for investigating lung vascular reactivity to this agent as described previously. The sequence of hypoxic and U46619 challenges was started 80–100 min after removal of the animals from the hypoxic chambers. For analysis, the maximum strength of the second hypoxic challenge and the first U46619 challenge was quantified. Normoxic vascular tone was assessed after the initial steady state period.

Measurement of exhaled NO

The technique for monitoring of exhaled NO of isolated rabbit lungs has been described previously. Briefly, an aliquot of the mixed expired gas was continuously forwarded to a chemiluminescence NO-analyzer (Sievers 280 NOA, Sievers Instruments, Boulder, CO, USA), and its NO quantity measured in ppb (parts per billion, v/v).

Vessel morphometry

For histological analysis, the isolated lungs were perfused with Zamboni’s fixative for 30 min at a pulmonary artery pressure of 15 mmHg and a left ventricular pressure of 2 mmHg. After ligation of the pulmonary artery and veins...
lungs were removed and placed in the Zamboni fixative for 6 h at room temperature. After incubation for 12 h at 4 °C in 0.1 M phosphate buffer, the tissue was dehydrated and infiltrated with paraffin wax in an automated vacuum tissue processor (Leica TP1050, Bensheim, Germany). Sections (3 μm) were stained with anti-α-smooth muscle actin antibody (dilution 1:900, clone 1A4, Sigma, St. Louis, MO) pretreated with trypsin (10 min, 37 °C). For detection, the Vectastain ABC Elite Kit Mouse IgG (Vector/Linaris, Wertheim-Bettingen, Germany) was used according to the manufacturer’s protocol. For visualization, the Vector VIP substrate kit for peroxidase (Vector/Linaris, Wertheim-Bettingen, Germany) was applied. Nuclear counterstaining was done with methyl green (Vector/Linaris, Wertheim-Bettingen, Germany). For determination of the degree of muscularization, vessels with a diameter ranging 80–150 μm were classified as non-muscular (no smooth muscle cells detectable with actin staining), partially muscularized (at least one smooth muscle cell up to 75% circumference with actin staining), and fully muscularized (≥75% of circumference with actin staining) as described previously.22 The analysis was done in the left upper, right lower and left lower lobe by counting 50 vessels from each lobe. The portion of the vessels from each category was calculated in percent of total vessel count. The morphometric analysis was done from sections blinded for the investigator.

Statistics
For calculation of statistical differences, analysis of variance with the Student–Newman–Keuls post hoc test was performed for comparison of more than two groups. For analysis of two groups, a Student’s t-test was applied. Statistical significance was assumed when p ranged <0.05. Data are given as mean ± standard error of the mean (SEM).

Results
Exposure of the animals to chronic normobaric hypoxia (10% O₂) for a period of 42 days resulted in a significant increase in the ratio of RV/(LV+septum) (Figure 1). In contrast to iloprost, treatment with NO significantly reduced the increase in right ventricular hypertrophy. The preventive effect of sildenafil was even more pronounced (Figure 1). A different profile was evident for pulmonary artery pressure measured in the intact animal as well as in the isolated lung preparation (Figure 2). When PAP was assessed in the intact animal only iloprost reduced PAP when compared to NO and sildenafil (Figure 2a). In isolated, blood-free, perfused lungs of these animals, no significant difference was detected between the groups treated with iloprost, NO, or sildenafil: all treatments only showed a tendency towards minor reduction of PAP under these conditions of in vitro perfusion (Figure 2b). The degree of muscularization of the pulmonary vasculature was significantly increased after chronic hypoxia. The portion of fully muscularized vessels was increased from 14.0 ± 4.0% to 43.5 ± 5.3% (n = 12). In parallel, the portion of the non-muscularized vessels decreased from 39.6 ± 4.8% to 12.4 ± 2.9%. The increase in the portion of the fully muscularized vessels was equally prevented in the iloprost and the sildenafil treated groups, but not in the NO treated group (Figure 3). The decrease in the portion of the non-muscularized vessels occurring during chronic hypoxia was not significantly affected by any of the treatment regimes. Iloprost increased the portion of partially muscularized vessels as compared to the hypoxic and normoxic control (Figure 3).

When explanted perfused and ventilated lungs were challenged with acute hypoxic ventilation maneuvers, a significant reduction in the strength of HPV from 2.2 ± 0.4 to 0.9 ± 0.1 mmHg (n = 12) was observed, as described previously.9 This decrease was virtually abrogated in the NO treated group, but not in the sildenafil- and iloprost-treated groups (Figure 4a). As previously described, chronic hypoxia downregulated HPV but not vasoconstrictions induced by the thromboxane mimetic U46619, excluding a general effect of chronic hypoxia on vascular contractility (Figure 4b).4 Only treatment with NO showed a tendency towards an increased response to vasoconstrictor challenges with U46619 (Figure 4b). Exhaled NO levels in the isolated perfused lung preparation were 109 ± 9 ppb and were not affected by exposure to chronic hypoxia (116 ± 7 ppb) or any of the treatment regimes. During acute hypoxic ventilation, a sudden decrease by 23 ± 2 ppb (n = 5–12 each) in exhaled NO levels occurred, persisting the entire hypoxic ventilation period as previously described.23 This decrease was also not
significantly affected by chronic hypoxia exposure (22 ± 2 ppb, \( n = 12 \)) exposure or any of the treatments.

**Discussion**

The present study explored the effects of inhaled NO, intravenous iloprost and oral sildenafil on right ventricular hypertrophy, pulmonary hemodynamics, structural changes of the pulmonary vasculature, and acute HPV in a rabbit model of hypoxia-induced pulmonary hypertension. Exposure of rabbits to 42 days of chronic hypoxia resulted in development of pulmonary hypertension with a significant increase in pulmonary artery pressure and pulmonary vascular resistance, right ventricular hypertrophy and an increased muscularization of distal pulmonary vessels. These effects are comparable to those found in other species such as mice and rats\(^8\) and are accordance with our recent investigation in this rabbit model.\(^4\)

**Figure 2** Pulmonary artery pressure assessed in vivo and in the isolated perfused and ventilated lung preparation. Pulmonary artery pressure was either assessed in the anesthetized animal (a) or in the ex vivo perfused and ventilated lung (b). Data are given for normoxic controls (normoxia), animals exposed to chronic hypoxia only, and chronic hypoxic animals treated with iloprost, NO, or sildenafil. Data from the isolated lung preparation are taken after the initial steady state period of lung perfusion. Data are from at least \( n = 5 \) animals per group. \( ^{*} p < 0.05, \; ^{**} p < 0.01, \; ^{***} p < 0.001 \) indicate significant differences compared to normoxic controls. \( ^{#} p < 0.05 \) indicates significant differences compared to hypoxic controls.

**Figure 3** Degree of muscularization in small pulmonary vessel. (a) The portion of fully muscularized, partially muscularized, and non-muscularized vessels (80–150 μm in diameter) is given in percent of total vessel count. \( ^{*} p < 0.05, \; ^{**} p < 0.01, \; ^{***} p < 0.001 \) indicate significant differences compared to normoxic controls. \( ^{#} p < 0.05 \) indicates significant differences compared to hypoxic controls. (b) Representative examples for immunostaining against α-smooth muscle actin in non (non), partially (partial), and fully (full) muscularized vessels.

hypertrophy, pulmonary hemodynamics, structural changes of the pulmonary vasculature, and acute HPV in a rabbit model of hypoxia-induced pulmonary hypertension. Exposure of rabbits to 42 days of chronic hypoxia resulted in development of pulmonary hypertension with a significant increase in pulmonary artery pressure and pulmonary vascular resistance, right ventricular hypertrophy and an increased muscularization of distal pulmonary vessels. These effects are comparable to those found in other species such as mice and rats\(^8\) and are accordance with our recent investigation in this rabbit model.\(^4\)

**General hemodynamic effects and structural changes of the pulmonary vasculature**

When comparing the effects in the different groups treated with inhaled NO, intravenous iloprost or oral sildenafil, sildenafil was the most effective agent in the prevention of right ventricular hypertrophy. However, this effect did not correlate with the effect of sildenafil on pulmonary artery pressure assessed in vivo. Elevation of PAP under chronic hypoxia was prevented in all of the treatment groups with
the most prominent effect observed for iloprost. A quite similar pattern was found when lungs were investigated in the isolated perfused lung preparation, where PAP directly reflects pulmonary vascular resistance. Under these conditions, the increase in pulmonary vascular resistance was less significant in all treatment groups with no differences between the different treatment regimes. The slight differences between the PAP measurements in vivo and in the isolated lung may be explained by (1) the fact that pulmonary artery pressure determined in the isolated lung is generally lower than in vivo, making it more difficult to discriminate changes with statistical significance and (2) by the fact that changes in cardiac output may affect PAP in vivo. Quantification of the degree of vascular muscularization revealed that not even this structural parameter indicative of pulmonary hypertension correlated with the effects of sildenafil, NO, and iloprost on right ventricular hypertrophy, PAP, or pulmonary vascular resistance. The increase in the degree of muscularization was prevented by sildenafil and iloprost, but not by NO; whereas sildenafil was the most effective agent in reducing right ventricular hypertrophy, and iloprost was the most effective agent in reducing PAP. These findings favorably support the idea that different mechanisms underlie the hemodynamic effects of the different treatment regimes, and that the vascular and right ventricular remodeling processes occurring in chronic hypoxia are not necessarily correlated with each other, or with the changes observed in hemodynamics. Such a view challenges previous observations, demonstrating that increases in pulmonary artery pressure correlate well with the development of right ventricular hypertrophy (e.g. 25,26). Although there is no doubt that an increase in pulmonary vascular resistance induces right ventricular hypertrophy, there is good reason to assume that right heart hypertrophy can be suppressed without a preceding reduction in pulmonary vascular resistance. In this regard, Takimoto et al.27 have shown that left heart hypertrophy can be reduced in a model of arterial banding. Such differential effects may be caused by different proproliferative mechanisms being active in right heart and vascular remodeling, which, in addition, may differentially be affected by the agents used for treatment in our study. In addition, our data suggest that hypoxia per se can induce right heart hypertrophy as both the degree of muscularization as well as the pulmonary vascular resistance was lowered by iloprost without significant reduction in right heart hypertrophy. Moreover, it has been questioned whether an increase in the degree of muscularization is sufficient per se to increase pulmonary vascular resistance.28 Thus, together with our study, there is good evidence that indeed such suggestions are valid for pulmonary hypertension. Even if our data cannot exclude that different dosages of the respective agents applied could have different effects on the different parameters of pulmonary hypertension, our data at least prove that the various parameters can be affected separately and that not all of them are necessarily linked. Future studies with a curative approach may resolve this if this is also true for reversal of pulmonary hypertension, when starting the treatment after establishment of hypoxia-induced pulmonary hypertension.

Comparing our data to previous studies, investigations in mice and rats revealed that treatment with oral sildenafil reduces the hypoxia-induced increase in right ventricular mass, pulmonary artery pressure and muscularization of small pulmonary arterial vessels.8,9,11 These findings are well in line with our observations.

For inhaled NO, a reduction in right ventricular hypertrophy and de novo muscularization induced by chronic hypoxia was reported.5,7 The failure of inhaled NO to
decrease pulmonary artery pressure measured in the isolated lung preparation is in line with the investigation of Frank et al.\textsuperscript{29} in rats. In contrast to the investigations in rats,\textsuperscript{6,7} the vascular remodeling process induced by chronic hypoxic exposure was not attenuated by inhaled NO in our experiments, although Kouyoumdjian et al.\textsuperscript{6} also used a concentration of NO as low as in our investigation. These differences may reflect species variations as to the role of NO in the vascular remodeling process.

Concerning the effects of iloprost, to the best of our knowledge, only one recent investigation exists, appearing while the manuscript for the present investigation was in preparation, which investigated the effects of this agent on the hypoxia-induced vascular remodeling processes and pulmonary hemodynamics.\textsuperscript{10} However, no significant effect was observed in this investigation in rats, although a 10-fold higher iloprost concentration was applied compared to our investigation, which again may reflect species differences. Studying the effect of iloprost on vascular remodeling is clearly important, since it has been shown that long-term therapy with iloprost improves exercise tolerance in patients with severe pulmonary arterial hypertension.\textsuperscript{30} This is in line with the effects of prostacyclin, shown to antagonize the development of hypoxia-induced pulmonary hypertension in mice chronically overexpressing PGI\textsubscript{2} synthase.\textsuperscript{5} Although iloprost is used as an aerosol for treatment of patients with pulmonary hypertension, we applied this agent intravenously to assure constant dosing. However, comparing the effectiveness of our study with the effect of inhaled iloprost in monocrotaline-induced pulmonary hypertension in rats suggests a similar effective dosage to be active in our investigation.\textsuperscript{31}

**Effects on hypoxic pulmonary vasoconstriction**

In addition to the basal hemodynamic changes, we assessed the vasoreactivity to acute hypoxic and pharmacological vasoconstrictor challenges. In this context, we have recently demonstrated that hypoxic vasoconstriction, but not vasoconstrictions induced by the thromboxane mimetic U46619, are downregulated in rabbits after exposure to chronic hypoxia,\textsuperscript{4} and that HPV is restored by chronic treatment with inhaled NO, which is suggested to be an unspecific effect resulting in amplified vasoreactivity.\textsuperscript{4} Beyond these investigations, we have now additionally investigated the effects of sildenafil and iloprost on the downregulation of HPV. Interestingly, only in the NO-treated group was the downregulation of HPV prevented, whereas treatment solely with sildenafil or with iloprost did not affect the attenuation of HPV. The lack of an effect of sildenafil suggests that the preservation of HPV by NO is probably not facilitated by stimulation of cGMP synthesis. Moreover, we have now demonstrated for the first time that stimulation of the cAMP axis by iloprost does not restore HPV after chronic hypoxia, again supporting the idea that the preservation of HPV by NO is independent of cyclic nucleotides. Finally, our data suggest that the prevention of the downregulation of HPV by NO is not related to changes in lung NO synthesis, as no change in exhaled NO occurred in the different treatment groups (exhaled NO has been proven to be a good indicator for vascular NO production in isolated blood-free perfused lungs).\textsuperscript{4,21}

In conclusion, we demonstrated (a) that the parameters characterizing development of hypoxia-induced pulmonary hypertension, namely pulmonary artery pressure, pulmonary vascular resistance, right ventricular hypertrophy, vascular remodeling, and changes in vasoreactivity, are not functionally linked in rabbits under chronic hypoxia, (b) that the downregulation of HPV occurring under conditions of chronic alveolar hypoxia can be prevented by inhaled NO but not by sildenafil and iloprost, which suggests that restoration of HPV by NO is unrelated to the second messengers of NO, cGMP, and (c) that iloprost treatment is particularly effective in preventing lung vascular remodeling and sildenafil in preventing right heart hypertrophy in hypoxic induced pulmonary hypertension in rabbits.

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