Pulmonary Microvascular Responses to Inhaled Prostacyclin, Nitric Oxide, and Their Combination in Anesthetized Cats

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Abstract: Using an X-ray television system on anesthetized cats, we directly measured internal diameter (ID) changes in identical small pulmonary vessels (100–1,100 μm ID) in response to inhalations of 25, 250, and 2,500 ng/kg/min aerosolized prostacyclin (PGI_2), 4 and 34 ppm nitric oxide (NO), and the combination of aerosolized PGI_2 and NO. We also compared ID changes during 250 ng/kg/min PGI_2 inhalation both with and without an N^G -nitro-L-arginine methyl ester (L-NAME, 30 mg/kg i.v.) pretreatment. In the arteries, inhaled PGI_2 increased 100–900 μm vessel ID in a dose-dependent manner but caused no significant, or only slight, ID increases in the vessels larger than this. The greatest ID increase ($\sim 22\%$) was in the 100–500 μm arteries in response to 2,500 ng/kg/min PGI_2 inhalation. PGI_2 also increased the ID of the veins (6–12%), but the results were not dose related. NO inhalation also resulted in non-uniform ID response patterns similar to PGI_2 with no sig-

nificant, or only minimal, ID increases of the arteries $>900 \mu\text{m}$. The simultaneous inhalation of 2,500 ng/kg/min PGI_2 and 34 ppm NO increased the arterial ID (maximum $\sim 34\%$) more than either drug alone and to almost the same extent as brought about by injected papaverine (2 mg/kg), a smooth muscle relaxant. Inhaled PGI_2 (250 ng/kg/min) decreased pulmonary arterial pressure and increased arterial ID to nearly the same extent with or without L-NAME pretreatment. These results indicate that inhaled PGI_2 and inhaled NO locally dilate 100–900 μm pulmonary arteries in a dose-dependent manner and with a similar ID response pattern, and that the combination of these drugs produces a more enhanced vasodilator effect compared to their separate effects and induces the maximum dilated states. The data also suggest that inhaled PGI_2 dilates these arteries directly, rather than via secondary release of endogenous NO. [Japanese Journal of Physiology, 49, 89–98, 1999]

Key words: aerosolized prostacyclin, nitric oxide, enhanced vasodilator effect, small pulmonary vessels, angiography.

Inhaled nitric oxide (NO) has been described as an effective pulmonary depressor in various experimental and clinical pulmonary hypertensive situations [1, 2]. Furthermore, we have recently provided direct evidence of the pulmonary vasodilator effects of inhaled NO by measuring pulmonary vascular internal diameter (ID) with an X-ray TV system [3].

Intravenous administration of prostacyclin (PGI_2), an arachidonic acid metabolite, has also been shown to decrease pulmonary arterial pressure [4] and increase the ID of small pulmonary arteries [5]. It has been applied in various clinical pulmonary hypertensive conditions [6]. However, systemic administration of PGI_2 has a depressor effect in both pulmonary and

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systemic circulation, usually leading to unfavorable systemic hypotension [7].

Recently, inhaled nebulized PGI₂ has been attracting a great deal of attention as a selective pulmonary depressor which does not affect the systemic circulation [8]. Until now, however, no direct measurement of the ID has been carried out to determine which pulmonary vascular segment actually dilates in response to inhaled nebulized PGI₂ or to reveal the differences between the vasodilator effects of inhaled NO and PGI₂. Moreover, the effects of the simultaneous inhalation of these drugs have also not been investigated, although a few trials have been conducted with a combination of intravenous PGI₂ and inhaled NO [9–11].

In this study, we investigated 1) the action sites of inhaled PGI₂ and NO in the identical small pulmonary vessels (100–1,100 μ m ID) of anesthetized cats by directly measuring their ID changes and 2) the vasodilator effects of the combined inhalation of PGI₂ and NO, and compared them with those of papaverine, a smooth muscle relaxant. In addition, we investigated the effect of a NO-synthase inhibitor on the vasodilation caused by inhaled PGI₂ in order to examine whether endogenous NO mediates the PGI₂-induced vasodilations [12].

METHODS

Experimental procedure and angiography.

The experiments were carried out using 13 cats (2.0–4.1 kg bw). They were anesthetized with pentobarbital sodium (35 mg/kg I.P.), and the level of anesthesia maintained with supplemental doses of pentobarbital sodium (2–3 mg/kg I.V.) at 0.5- to 1-h intervals. Each cat was intubated with an endotracheal tube and artificially ventilated with room air at a tidal volume of 10–12 ml/kg and a rate of 20–24 cycles/min. The anesthesia and preparation of animals were performed in accordance with the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan.

A 4-F Swan-Ganz balloon-tip catheter (Edwards Labo.) was introduced fluoroscopically from the right jugular vein into the left main pulmonary artery. Two additional polyethylene catheters were also inserted into the right femoral artery and vein. The left-side rib cage (ribs 6–8) was partially excised to directly expose the left lower lobe to the X-ray. The end-expiratory pressure was adjusted to 4.0 cmH₂O to prevent lung collapse. Heparin was administered intravenously at a dose of 500 IU/kg to prevent blood coagulation in the catheters.

The system and the experimental setup for angiography have been described in detail previously [13]. In short, the cat was positioned within the X-ray apparatus box (Osaka Softex) and fixed so that the exposed left lower lung was automatically in contact with a plate just above the beryllium faceplate of an X-ray sensitive, 1-in. vidicon camera (Hamamatsu Photonics). The cat was partially covered with a heating pad to keep its rectal temperature at 37.5°C. The temperature in the box was maintained at 25–28°C, and the surface of the exposed lung was kept wet with warm (37°C) saline. During the temporary cessation of ventilation at end-expiration, a contrast medium (3–3.5 ml of 60% Urografin, Schering) [14] was injected into the left main pulmonary artery through the catheter at a constant speed (2.0 ml/s). Before and during the injection of contrast medium, the fluoroscopic image was monitored on a television and recorded at a high speed of 30 frames/s on a videocassette recorder (PWW-2800, Sony). Blood gases and pH were frequently measured throughout the experiment by a blood gas analyzer (ABL2, Radiometer). Systemic arterial blood gases were PaO_2 101 $\pm$ 4 Torr, $PaCO_2$ 30 $\pm$ 1 Torr, and pH 7.39 $\pm$ 0.01 under the baseline condition. These parameters were not significantly changed in response to PGI₂ and NO inhalations and papaverine injection.

Measurement of ID. The serial angiograms recorded on the videocassette recorder were transferred to a digital image processor (TVIP-2000, Nippon Avionics). To obtain the angiogram for measuring ID, four serial frames, in which vascular trees were extensively filled with contrast medium, were added up and averaged by the digital image processor. This was done for both pulmonary arteries and veins. The processed image was clearly copied onto paper with an imaging hard copy unit (model 4634, Sony Tektronix), and the ID of the pulmonary vessels on the copy was measured manually using a digitizer (model 9874A, Hewlett-Packard) connected to a minicomputer.

Analysis of ID response. Following the method described in our previous study [15], a random selection of many vascular sites was made for the ID measurement. The ID percent change in response to inhaled NO and PGI₂ was calculated against the initial baseline value at each measured vascular site. The measured sites were classified into three or five vascular groups with different ID sizes according to their initial baseline ID size.

Experimental protocol. Protocol 1: pulmonary vascular responses to inhaled PGI₂ and NO: The baseline angiogram was recorded first, followed

by the angiograms during 1) a nebulized glycine buffer inhalation, and 25, 250 and 2,500 ng/kg/min nebulized PGI₂ inhalations, 2) 4 and 34 ppm NO inhalations, 3) simultaneous inhalation of 2,500 ng/kg/min PGI₂ and 34 ppm NO, and 4) a smooth muscle relaxant, papaverine hydrochloride (2 mg/kg, I.V.). In four of eight cats, the experiments were carried out in a 1)→2)→3)→4) order; and for the other four cats, in a 2)→1)→3)→4) order. There was an interval of more than 1 h between steps 1) and 2), and between steps 3) and 4). We confirmed that ID changes returned to their initial baseline level after these intervals.

The periods of NO and PGI₂ exposure were 10 min, because both substances brought about an immediate decrease in pulmonary arterial pressure which reached its lowest point within 10 min. In the preliminary study, we found that the ID changes in response to 4–34 ppm NO inhalations returned to their initial baseline level ~10 min after the inhalations ceased, and those in response to 25–2,500 ng/kg/min PGI₂ inhalations returned ~15 min after discontinuance. Therefore, an interval of ~20 min between each inhalation was allowed to eliminate any influence of the preceding exposures. The angiogram with papaverine was obtained when pulmonary arterial pressure reached nearly the minimal level at 2–3 min after the injection ended.

Protocol 2: pulmonary vascular responses to inhaled PGI₂ with and without NO-synthase inhibition: Five of the thirteen cats were used in this protocol. The first paired angiograms were taken before and 10 min after the 250 ng/kg/min PGI₂ inhalation. PGI₂ was administered in the same manner as for protocol 1. N^ω-nitro-L-arginine methyl ester (L-NAME, 30 mg/kg I.V., Sigma) was injected after checking that ID changes had returned to their initial levels. The angiogram with L-NAME was obtained 20 min after the injection, followed by the angiogram with L-NAME plus 10 min periods of PGI₂ (250 ng/kg/min) inhalation.

Application of PGI₂. For the inhalation of PGI₂ in aerosol form, an ultrasonic nebulizer (NE-U11B, Omron, Kyoto, Japan) was connected between the ventilator and endotracheal tube. This nebulizer delivers aerosolized particles with an average diameter of 5 μm, which can therefore easily reach the lower respiratory tract. PGI₂ was supplied as a sodium salt (Wako Pure Chem. Ind., Japan) dissolved in a glycine buffer of pH 10.6 at 10, 100, and 1,000 μg/ml concentrations. These solutions were kept frozen at –20°C until use. They were thawed on ice and diluted 10 times with 0.9% saline just before administration. Twenty milliliters of solution was poured into the neb-

ulizer chamber.

Before the experiment, we estimated the amount of PGI₂ to be administered by connecting a water trap containing moisture absorbents to an endotracheal tube, in place of the animal, and weighing the fluid which was absorbed after nebulization. Approximately 25 ng/kg/min PGI₂ was delivered with this system when 1 μg/ml PGI₂ was prepared in the chamber.

Application of NO. NO was delivered from a source tank of 812 ppm in N₂ (Takachiho, Tokyo, Japan). With the flow controller, a given concentration of NO at 4 and 34 ppm was obtained by mixing with N₂ and O₂ while inspired O₂ concentration (FiO₂) was maintained at 21%. The NO gas mixture was supplied to the inspiratory circuit of the ventilator via a 1-l reservoir bag. The NO gas mixture passed through a tube filled with soda-lime to scavenge NO₂ just before the inlet of the ventilator. The concentrations of the inspired NO/NO₂ were monitored at the endotracheal tube using a chemiluminescence NO/NO₂ analyzer (ECL-88US, Yanako, Kyoto, Japan). The inspired NO concentration decreased by less than 1 ppm when PGI₂ nebulizer was added to 34 ppm inhaled NO. The inspired NO₂ concentration was under 0.3 ppm and did not change even when the nebulization was added.

Methemoglobin levels were 1.0±0.1% during 4 ppm NO, and increased to 1.3±0.2% during 34 ppm NO ($p<0.05$), but there was no difference in the methemoglobin level between 34 ppm NO inhalation by itself and its combination with nebulized 2,500 ng/kg/min PGI₂.

Contribution of pressure- and flow-sensing mechanisms to ID changes in response to agents. In this study, mean pulmonary arterial pressure (PAP) was decreased by 2–4 mmHg during PGI₂, NO, and PGI₂+NO inhalations, and papaverine injection. It was therefore possible that the local pressure-sensing mechanism (see DISCUSSION) influenced the ID change pattern caused by these agents. To examine this possibility, we preliminarily measured the effect of mechanically-induced PAP decrease on the ID of small pulmonary vessels in four cats. Partial interruption of pulmonary blood flow with a balloon-tip catheter inserted into the vena cava inferior lowered PAP by ~4 mmHg below the baseline level, but caused no significant change in the small-vessel ID. This suggests that the possible influence of this mechanism is small.

We also examined the possibility that the local flow-sensing mechanism (see DISCUSSION) participated significantly in the ID changes caused by inhaled PGI₂ and NO, and injected papaverine. To do this, further preliminary experiments were carried out

Table 1. Hemodynamic data.

Condition	PAP (mmHg)		SAP (mmHg)	
	Before administration	During administration	Before administration	During administration
Protocol 1 (<i>n</i> =8)				
Aerosolized PGI ₂				
25 ng/kg/min	17.4±0.7	15.4±0.7*	110±3	108±3
250 ng/kg/min	16.8±0.9	14.5±0.8*	106±4	97±4*
2,500 ng/kg/min	16.3±0.9	13.5±0.9*	101±2	91±4*
NO				
4 ppm	17.3±1.0	15.0±1.5*	104±7	103±8
34 ppm	16.8±1.1	13.7±1.2*	105±7	102±8
2,500 ng/kg/min PGI ₂ +34 ppm NO	16.4±0.8	13.1±1.0*	100±3	89±5*
Papaverine	17.5±1.0	14.0±1.1*	97±8	68±9**
Protocol 2 (<i>n</i> =5)				
Aerosolized PGI ₂				
250 ng/kg/min	18.2±0.5	without L-NAME pretreatment 15.4±0.6*	104±3	95±6*
250 ng/kg/min	21.0±1.0	with L-NAME pretreatment 16.8±1.1*	121±7	112±10

Values are means±SE; *n*, number of animals; PAP, mean pulmonary arterial pressure; SAP, mean systemic arterial pressure. Significant difference from the condition before administration: * *p*<0.05, ** *p*<0.01.

using the following two protocols in another four cats. Initially, changes in local blood flow velocity (*V*) in the small pulmonary arteries were measured in response to PGI₂ and NO inhalations, and papaverine injection. *V* was obtained by measuring the distance that the leading edge of the contrast medium moved per 0.1 s [13] in ~400–600 μm arteries. We found that mean percent decreases in *V* were 11, 11, 5, and 8% in response to PGI₂ (2,500 ng/kg/min), NO (34 ppm), and 2,500 ng/kg/min PGI₂+34 ppm NO inhalations, and papaverine (2 mg/kg) injection, respectively. Secondly, the effect of mechanically-induced decrease in *V* on the ID of ~400–600 μm pulmonary arteries was examined. *V* was decreased by 20–30% without PAP decrease when blood flow to the left lung was partially interrupted. We found no significant ID changes in response to such *V* decreases. From these data, we considered the contribution of the flow-mediated response to the present ID change to be small.

Statistical methods. Single comparisons were made as appropriate using Student's paired *t*-test, and multiple comparisons in hemodynamic data and ID responses for the different doses and drugs were made using analysis of variance followed by Tukey's multiple comparison test. ID response differences among the five vascular groups with different ID sizes were assessed in the same way. Significance was defined as *p*<0.05. All results are expressed as means±SE.

RESULTS

1. Responses to inhalations of PGI₂, NO, and their combination

Hemodynamic data. The values of PAP and mean systemic arterial pressure (SAP) before and during the inhalation of PGI₂, NO, and their combination are summarized in Table 1. These parameters were obtained just before injection of the contrast medium. With 25, 250, and 2,500 ng/kg/min PGI₂ nebulization, PAP decreased significantly by 2.0, 2.3, and 2.8 mmHg, respectively. With 4 and 34 ppm NO inhalations, PAP also decreased significantly by 2.3 and 3.1 mmHg, respectively. During the inhalations of 250 and 2,500 ng/kg/min PGI₂, SAP decreased significantly by 9 and 10 mmHg, respectively. On the other hand, NO inhalations caused no significant changes in SAP.

The combined inhalation of 2,500 ng/kg/min PGI₂ and 34 ppm NO decreased both PAP and SAP by 3.3 and 11 mmHg, respectively. In combination, they had a tendency to decrease PAP more than when administered individually, but this difference was not statistically significant. Papaverine injection significantly decreased PAP and SAP by 3.5 and 29 mmHg, respectively.

ID responses to PGI₂ and NO inhalations.

Figure 1 shows typical ID changes in the small pul-

Fig. 1. Typical angiograms of small pulmonary arteries obtained before (top left) and during (top right) aerosolized 2,500 ng/kg/min PGI₂ inhalation, 34 ppm NO inhalation (bottom left), and their simultaneous inhalation (bottom right) in the same cat.

monary arteries in response to inhalations of 2,500 ng/kg/min PGI₂, 34 ppm NO, and their combination in the same cat. With PGI₂, and also with NO inhalation, the ID in many arterial branches obviously increased. When they were combined, the ID clearly increased more than when they were inhaled separately.

The relationship between vessel size and percent ID change in the small arteries and veins is shown in response to PGI₂ inhalations (Fig. 2A) and NO inhalations (Fig. 2B). During 25 ng/kg/min PGI₂ inhalation, the ID of 100–500 μ m arteries increased significantly. During the inhalation of 250 and 2,500 ng/kg/min PGI₂, the ID of these arteries increased further and the larger arteries (500–900 μ m) also dilated. However, the arteries >900 μ m showed no significant increase in ID at any dose less than 2,500 ng/kg/min. The highest dose increased the ID minimally but significantly. The maximum ID increase (~22%) occurred in the 100–500 μ m arteries during 2,500 ng/kg/min PGI₂ inhalation. In the veins, the ID of 100–700 μ m vessels increased during 25 and 250 ng/kg/min inhalation. During 2,500 ng/kg/min PGI₂ inhalation, the ID of 700–1,100 μ m veins also increased. However, the

dose-response pattern was not as clear as in the case of arteries.

In response to the inhalation of a glycine buffer, which was used as a PGI₂ solvent, neither the hemodynamic parameter nor the ID changed significantly.

NO inhalation also increased the ID of the small arteries and veins. In the arteries, dose-related ID increases were induced and the response pattern in the serially connected vessels was similar to that observed for inhaled PGI₂. The arteries >900 μ m showed no significant increase in ID with 4 ppm NO and only a minimal, but significant, increase with 34 ppm NO, whereas the ID of 100–900 μ m arteries increased significantly with the two doses of NO. The inhaled 34 ppm NO increased the ID of these arteries to nearly the same extent as inhaled 2,500 ng/kg/min PGI₂, with the maximum increase (~25%) in the 300–500 μ m arteries. In the veins, the 100–700 μ m vessels displayed significant ID increases, but the larger vessels did not.

The relationships between vessel size and ID percent change are compared between PGI₂ and NO, and their combined inhalation (Fig. 3A). In the arteries

(A) *Inhaled aerosolized PGI₂* (B) *Inhaled NO*

Fig. 2. Percent changes in internal diameter (ID) at different serial segments of small pulmonary arteries and veins in response to aerosolized PGI₂ (A) and NO (B) inhalations. Numbers of measured sites in five vascular groups, i.e., 100–300, 300–500, 500–700, 700–900, 900–1,100 μm, are 105, 103, 73, 40, and 30, respectively, in the arteries. They are 74, 73, 39, 28, and 22, respectively, in the veins. Significant ID increase from baseline value: **p*<0.05, ***p*<0.01.

Fig. 3. Percent ID changes in response to the combined inhalation of 2,500 ng/kg/min PGI₂ and 34 ppm NO are compared with those during their separate inhalations (A), and with those after papaverine injection (B). Numbers of measured sites in five vascular groups are the same as those in Fig. 2. **p*<0.05, ***p*<0.01 compared with NO alone; †*p*<0.01 compared with PGI₂ alone.

Fig. 4. Absolute ID changes at different serial segments of pulmonary arteries in response to aerosolized 250 ng/kg/min PGI₂ inhalations with and without L-NAME pretreatment. Number of measured sites in three vascular groups, i.e., 100–400, 400–700, 700–1,100 μm, are 76, 79, and 75, respectively.

ranging from 100 to 900 μm, the combination of 2,500 ng/kg/min PGI₂ and 34 ppm NO inhalations increased the ID more than either drug alone. However, such enhanced effects were not seen in arteries larger than this. The maximum ID increase (~34%) in response to the combined inhalation was observed also in the 300–500 μm arteries.

The magnitudes of ID increase are compared between the simultaneous inhalation of 2,500 ng/kg/min PGI₂ and 34 ppm NO, and the injection of papaverine (Fig. 3B). There were no significant differences between them in 100–900 μm arteries and 100–1,100 μm veins. In the 900–1,100 arteries, however, the ID increase due to papaverine injection was significantly greater than that due to simultaneous inhalation.

2. Responses to PGI₂ with and without pretreatment of L-NAME

Hemodynamic data. The effects of 250 ng/kg/min PGI₂ inhalations with and without L-NAME pretreatment are shown in Table 1. Both PAP and SAP increased in response to L-NAME injection by 2.8 and 17 mmHg, respectively. The 250 ng/kg/min PGI₂ inhalation with L-NAME decreased PAP to nearly the same extent as that without L-NAME.

ID responses. On average, the L-NAME injection decreased the ID of arteries to ~87% of the initial level. The absolute values of the arterial ID increases in response to 250 ng/kg/min PGI₂ inhalation were not significantly different between the conditions with and without L-NAME pretreatment (Fig. 4).

DISCUSSION

Using an X-ray TV system [13], we quantitatively measured the effects of inhaled PGI₂, NO and their combination on the ID of identical feline small pulmonary vessels (100–1,100 μm ID) *in vivo*. In addition, we investigated the effects of NO-synthase inhibition on ID response to inhaled PGI₂.

Action sites of inhaled PGI₂ and NO. PGI₂ has been shown to decrease pulmonary and systemic arterial pressure in a dose-related manner when administered by injection [4, 16]. But with this systemic depression, an increase in intrapulmonary shunt and hypoxemia limit the clinical application of PGI₂ [8]. It was to overcome these demerits that aerosolized inhaled PGI₂ was designed [8]. Previous studies have shown that inhaled PGI₂, like inhaled NO, is an effective depressor for pulmonary hypertension not only in experimental models of alveolar hypoxia [8, 17] and acute respiratory failure [18], but also in clinical cases of acute respiratory distress syndrome in adults [19–23] and children [24], and primary pulmonary hypertension [7] and congenital heart disease in newborns [25]. However, the difference in the action sites of PGI₂ and NO in the serially connected pulmonary vessels has not been clarified.

In this study, we directly measured ID changes in feline small pulmonary vessels (100–1,100 μm), which have rich smooth muscle layers [26, 27], in response to the inhalation of PGI₂ and NO. We found that, in the arteries, 25–2,500 ng/kg/min PGI₂ inhalations caused non-uniform ID increases within the serially connected vessels and dose-related ID increases at the different serial segments (Fig. 2A). Although 25 ng/kg/min PGI₂ inhalations only increased the ID of 100–500 μm arteries, 2,500 ng/kg/min PGI₂ acted on arteries over a wider range (100–900 μm) and with greater effect. However, the responses in 900–1,100 μm arteries were either very small or nonexistent. We also found that NO inhalation caused non-uniform ID increases in patterns similar to PGI₂ (Fig. 2, A and B); no significant, or only minimal, ID increases were induced in the arteries >900 μm. Intravenous infusion of papaverine, a smooth muscle relaxant, dilated the arteries >900 μm more greatly than inhaled PGI₂, NO, and their combination (Fig. 3, A and B). Therefore, the minimal vasodilation in the larger arteries cannot be ascribed to a diminution in relaxant ability of vascular smooth muscles in the region of 1,000 μm in diameter. One possible reason is the failure of inhaled NO and PGI₂ to directly diffuse into the smooth muscle cells of the arteries >900 μm.

Inhaled PGI₂ (25–250 ng/kg/min) and NO signifi-

cantly increased the ID of 100–700 μm veins, but the ID increase was not dose-related, in contrast to the arterial response. The reasons for the difference in the dose-response pattern between the artery and vein remain to be elucidated. However, papaverine increased the venous ID far less than the arterial ID (Fig. 3B), suggesting more dilated states in the vein than in the artery under baseline conditions. One of the reasons may be a difference in initial vascular tone between these vessels.

Inhaled PGI_2 is used as a selective pulmonary depressor which does not affect the system circulation. However, a few previous studies have reported that a large dose of inhaled PGI_2 also decreased [21], or had a tendency to decrease [17], systemic vascular resistance. In our study, significant SAP decreases were observed in response to 250 and 2,500 ng/kg/min PGI_2 (Table 1). In addition, 2,500 ng/kg/min PGI_2 increased the ID of the larger veins (700–1,100 μm), although the smaller doses of PGI_2 did not (Fig. 2). During NO inhalations, in contrast, similar effects were not observed. Such differences may be explained by the data showing that NO becomes inactive within seconds due to binding tightly to hemoglobin [28], while PGI_2 has a longer half-life (2–3 min) and does not become inactive while passing through the lung [29, 30], therefore, it may “spill over” [19] into the pulmonary veins and systemic vessels.

Blood vessels are sensitive to local changes in intraluminal pressure [31] and flow velocity (V) [32, 33]. The pressure-dependent response to a rapid change in transmural pressure has been reported to take 15–30 s to become fully established [31]. The flow-mediated response reaches a maximum in ~ 50 s after the onset of V increase [32, 33]. In this study, there were PAP decreases of 2–4 mmHg (Table 1) and V decreases of 5–11% (see METHODS) when the angiograms were recorded ~ 10 min after PGI_2 , NO, or PGI_2 +NO inhalation, and ~ 3 min after papaverine injection. It is therefore possible that the pressure- and flow-mediated responses became fully established and influenced the ID change caused by these agents. However, we found no significant ID changes when PAP was mechanically decreased by ~ 4 mmHg and V by 20–30% (see METHODS). This suggests that the possibility is small and that direct actions of these substances on the small pulmonary vessels are the primary cause of the present ID change.

Enhanced dilator effect of inhaled PGI_2 and NO on pulmonary arteries. A few previous studies have reported that the combination therapy of intravenous PGI_2 and NO inhalation was more effective in lowering PAP than either drug by itself in a

neonate with persistent pulmonary hypertension [9] and congenital aortic stenosis [10], and in pigs with pulmonary hypertension [11]. However, the effects of an inhaled PGI_2 and NO combination on the pulmonary vessels was still to be investigated.

In this study, we first demonstrated that the simultaneous administration of inhaled 2,500 ng/kg/min PGI_2 and 34 ppm NO induced greater ID increases in small pulmonary arteries (100–900 μm) than when the drugs were administered separately (Fig. 3A). The inhalation of 5–40 ppm NO has been reported to induce the maximum pulmonary depressor effect [11, 17]. In the preliminary experiments, we confirmed that 34 ppm NO inhalation increased ID to almost the same extent as 84 ppm NO inhalation. The inhalation of 2,500 ng/kg/min PGI_2 would have extensively activated the pulmonary vessels in our preparations, because it did spill over into the systemic circulation to decrease SAP significantly (Table 1). These data mean that, when PGI_2 inhalation was added to the maximally effective dose of inhaled NO, even further vasodilation of the small arteries occurred. This more enhanced vasodilator effect, compared with the separate effects, may be explained by the fact that these agents relax the vascular smooth muscles through two different second-messenger systems; PGI_2 stimulates the cAMP pathway, whereas NO activates the cGMP pathway [34, 35]. We also found that the combination of 2,500 ng/kg/min PGI_2 and 34 ppm NO inhalations increased the ID of the small arteries and veins almost as much as papaverine (2 mg/kg). Preliminary dose response experiments showed that the maximum response is achieved with this dose of papaverine. These findings suggest the possibility that this combination is able to induce maximum dilated states in small pulmonary vessels. It is noteworthy that papaverine relaxes vascular smooth muscles by inhibiting cyclic nucleotide phosphodiesterases and accumulating both cGMP and cAMP [36, 37].

The combination of PGI_2 and NO inhalations may be applicable to clinical cases. In the three previous studies on the combination of intravenous PGI_2 and inhaled NO [9–11], there was an inevitable decrease in SAP because of the intravenous PGI_2 effects on systemic circulation. However, nebulized PGI_2 inhalation at an appropriate dose can spare this unfavorable effect, and its combination with inhaled NO seems to be a better selective pulmonary vasodilator than the combination of intravenous PGI_2 and inhaled NO.

Inhaled PGI_2 dilates small pulmonary arteries with its own direct effect. Using pig vessels, Shimokawa *et al.* have showed that PGI_2 relaxation is potentiated by the presence of the vascular en-

endothelium and that this phenomenon is heterogeneous: endothelium-dependent potentiation being clearest in coronary and basilar arteries but not in the carotid artery [12]. They have suggested that prostacyclin not only acts directly on the vascular smooth muscle cells but also stimulates endothelium-derived relaxing factor (EDRF) release. Other researchers have reported that exogenously administered PGI₂ does not cause endothelium-dependent relaxation in canine intrapulmonary arteries [38] or in the pig aorta [39].

To examine whether endogenous NO is involved when inhaled PGI₂ dilates the small pulmonary arteries *in vivo*, we investigated the effect of inhaled PGI₂ during NO-synthase inhibition. The L-NAME administration *per se* elevated PAP significantly (Table 1) and decreased the ID of the small pulmonary arteries to ~87% on average, which suggests that endogenous NO affects the basal vascular tone in pulmonary arteries. However, inhaled 250 ng/kg/min PGI₂ with L-NAME pretreatment decreased PAP just as effectively as without it (Table 1). Likewise, no significant difference was observed in ID responses to 250 ng/kg/min PGI₂ with or without L-NAME (Fig. 4). These data suggest that PGI₂ dilates small pulmonary arteries mainly via direct action on the vascular smooth muscle, rather than via the endothelial release of NO stimulated by PGI₂. This is consistent with the fact that adding inhaled PGI₂ to the maximally effective dose of inhaled NO caused further vasodilation of the small pulmonary arteries (Fig. 3A).

In summary, the results indicate that, in anesthetized cats, both inhaled PGI₂ and inhaled NO locally dilate small pulmonary arteries ranging from 100 to 900 μ m ID. These drugs caused similar non-uniform vasodilator patterns in serially connected arteries and dose-related dilations at different serial segments, the maximum dilation being in ~100–500 μ m arteries. An enhanced vasodilator effect was obtained when they were administered simultaneously. Moreover, the data suggest that inhaled PGI₂ dilates the pulmonary arteries mainly via its direct effect on the vascular smooth muscle, rather than via secondary NO release.

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