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ORIGINAL ARTICLE

Effect of carbon dioxide inhalation on pulmonary hypertension induced by increased blood flow and hypoxia

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Abstract There is now increasing evidence from the experimental and clinical setting that therapeutic hypercapnia from intentionally inspired carbon dioxide (CO_2) or lower tidal volume might be a beneficial adjunct to the strategies of mechanical ventilation in critical illness. Although previous reports indicate that CO_2 exerts a beneficial effect in the lungs, the pulmonary vascular response to hypercapnia under various conditions remains to be clarified. The purpose of the present study is to characterize the pulmonary vascular response to CO_2 under the different conditions of pulmonary hypertension secondary to increased pulmonary blood flow and secondary to hypoxic pulmonary vasoconstriction. Isolated rat lung ($n = 32$) was used to study (1) the vasoactive action of 5% CO_2 in either N_2 (hypoxic-hypercapnia) or air (normoxic-hypercapnia) at different pulmonary arterial pressure levels induced by graded speed of perfusion flow and (2) the role of nitric oxide (NO) in mediating the pulmonary vascular response to hypercapnia, hypoxia, and flow-associated pulmonary hypertension. The results indicated that inhaled CO_2 reversed pulmonary hypertension induced by hypoxia but not by flow alteration. Endogenous NO attenuates hypoxic pulmonary vasoconstriction but does not

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augment the CO_2-induced vasodilatation. Acute change in blood flow does not alter the endogenous NO production.

Introduction

There have been contradictory reports indicating that carbon dioxide (CO_2) may constrict, dilate, or have no action on the pulmonary vessels. Respiratory alkalosis (hypocapnia) produced by mechanical hyperventilation is a therapeutic strategy for persistent pulmonary hypertension in infants. Previous studies have indicated that respiratory alkalosis improves oxygenation and decreases the mean pulmonary arterial pressure (PAP) when the arterial pH is greater than 7.55 and the $PaCO_2$ is less than 22 mmHg [1]. On the other hand, permissive hypercapnia with a small tidal volume has been adapted to avoid ventilator-induced lung injury in patients with lung injury or acute respiratory distress syndrome. Such "protective" ventilator strategies minimize lung stretch and patient mortality and often lead to an elevation in $PaCO_2$ [2–4].

There is evidence that high CO_2 tension with elevated hydrogen ion concentration (low pH) in the blood increases the extracellular Ca^{2+} influx and accounts for the vasoconstrictory property of CO_2 in pulmonary circulation [5,6]. Nonetheless, CO_2 also plays a vasodilator role under the condition of high vascular tone induced by drugs or hypoxia, and such a vasodilatory effect is related to the concentration of inhaled CO_2, not to the blood pH value [7–11]. More recently, the potential beneficial effects of therapeutic hypercapnia as a result of direct improvements in gas exchange, anti-inflammatory events, and attenuation of ischemia-reperfusion, endotoxin, and ventilator-induced lung injuries have been reported in several studies [12–19]. Most previous studies indicate that the vasoactive action of CO_2 is dependent on the initial PAP; during basal tone condition, CO_2 is a mild vasoconstrictor, whereas at high pulmonary vascular resistance, it is a potent vasodilator [5,7–11,14,18]. The differences in pulmonary vascular tone may account for the discrepant vasoactive action of CO_2.

Our previous data represented the first demonstration of a pressure-response relationship between the degree of CO_2-induced vasodilatation and the level of PAP. We observed that the vasodilatory effect of CO_2 tends to be more evident at high PAP. In clinical settings, pulmonary arterial hypertension occurs under different clinical conditions depending on the associated disease. In our study, we also found that CO_2 is not a specific eliminator to hypoxia and endothelin-1 (ET-1)–induced pulmonary hypertension [20]. Because pulmonary hypertension secondary to increased pulmonary blood flow and pulmonary vasoconstriction often coexists with altered vascular reactivity, there has been considerable interest in the discrepant vasoactive action of inhaled CO_2 under the different conditions of pulmonary hypertension.

It is known that endothelial cells release vasoactive substances in modulating pulmonary vascular tone. Nitric oxide (NO) and ET-1 have been shown to be the major endothelium-dependent vasomediators [21–23]. The response of the pulmonary endothelium to increased flow and hypoxia may play an important role in the modulation of pulmonary vessel response to increasing pressure. There is evidence that NO plays an important role in the modulation of endothelial stress induced by increased pulmonary blood flow and hypoxic stimulation [24–27].

In the present study, we attempted to characterize the effect of inhaled CO_2 on pulmonary vascular response under different conditions of pulmonary hypertension induced by increased pulmonary blood flow and hypoxic pulmonary vasoconstriction. We also attempted to clarify the role of NO in mediating the pulmonary vascular response to hypercapnia, hypoxia, and increased pulmonary blood flow. Therefore, the pulmonary vascular responses to CO_2 inhalation were observed in isolated rat lungs under different levels of PAP induced by graded perfusion flow speed. The effects of inhaled CO_2 on pulmonary hypertension were evaluated by comparing vascular tone at normoxic-hypercapnia (5% CO_2 in air) and vascular tone at hypoxic-hypercapnia (5% CO_2 in N_2) ventilation. Furthermore, to clarify the modulatory role of NO, we investigated the effect of NO and ET_B (endothelin type B receptor) blockade on hypercapnia, hypoxia, and increased blood flow induced changes in pulmonary vascular tone.

Materials and methods

Animals

Adult male Sprague-Dawley rats weighing 300–350 g were used. The specific pathogen-free animals were purchased from the National Animal Center and housed in a temperature-controlled animal room. The room temperature was maintained at 22 ± 1°C under a 12/12-hour light/dark regimen. Food and water were available *ad libitum*. The use and care of the animals were approved by the Animal Care and Use Committee of Kaohsiung Medical University.

Isolation and perfusion of rat lungs

The rats were deeply anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg). The experimental setup was modified from previous studies [28]. After tracheotomy, the lungs were artificially ventilated with room air. Heparin (1 U/g) was administered into the left ventricle after a midsternal thoracotomy. A total of 10 mL of blood was collected from the right ventricle and mixed with 10 mL of Hank's balanced salt solution (in mM: NaCl 136.9, KCl 5.4, glucose 5.6, KH_2PO_4 0.4, Na_2HPO_4 0.3, and 6% albumin and pH was adjusted to 7.35–7.40) and subsequently used to perfuse the isolated lungs. Furthermore, the perfusion medium was gassed with a mixture of 5% CO_2 and monitored continuously for pH. During the initial stabilization period, the pH was adjusted to 7.4 ± 0.05 with HCl. A cannula was placed in the pulmonary artery through a puncture into the right ventricle, and a tight ligature was placed around the main trunk of the pulmonary artery. A large catheter was inserted into the left atrium through the left ventricle and mitral valve and fixed by ligature at the apex of the heart to divert pulmonary venous outflow into a reservoir. A third ligature was placed above the arterio-ventricular junction to prevent perfusate flow into the ventricles. Perfusion fluid maintained at 37 ± 0.5°C was circulated by use of a roller pump at a flow rate of 10 mL/min. The PAP and pulmonary venous pressure were measured with pressure transducers (Gould Instruments, Cleveland, OH, USA) from a side arm of the inflow and outflow cannula. The pulmonary venous pressure was set at 2 mmHg by adjusting the height of the venous reservoir.

After an initial hyperinflation to reverse atelectasis, the lungs were ventilated at 60 breaths/min and tidal volume at 2.5–3 mL. The end-expiratory pressure was set at 2 cmH_2O. The gas tension in the perfusate was measured at the beginning of each experiment and after changes in ventilatory gas mixtures, by collecting perfusion fluid anaerobically and analyzing immediately using a gas analyzer (Stat Profile 5; Nova Biomedical, MA, USA). There were three criteria for a satisfactory isolated lung preparation: no leakage at the site of cannula insertion, no evidence of hematoma or edema, and an isogravimetric state.

Drug preparation and delivery

N-Nitro-L-arginine methyl ester (L-NAME, NO synthase blocker) and BQ788 (ET_B receptor blocker) were purchased from Sigma (Sigma Chemical, St. Louis, MO, USA) and were added to the venous reservoir to give a final concentration of 400 mM and 1 μM in the perfusate.

Experimental outline

Experiments in isolated perfused lungs were carried out in two series; and different perfusion rates (13, 18, 25 mL/min) were used to induce various levels of PAP elevation. In Experiment Series A, we examined the effect of CO_2 on the flow-associated PAP elevation under normoxic-hypercapnia ventilation both with (Group A1) and without (Group A2) endogenous NO. To assess the role of NO in mediating the pulmonary vascular response to flow challenge and hypercapnia, Group A2 was pretreated with L-NAME (400 mM) and BQ788 (1 μM); and the PAP change was compared among Groups A1 and A2.

Experiment Series B was carried out to evaluate the effect of CO_2 on the flow-associated PAP elevation under hypoxic-hypercapnia ventilation with (Group B1) and without (Group B2) endogenous NO. To clarify the modulation role of NO in response to flow challenge and hypoxic-hypercapnia, Group B2 were pretreated with L-NAME (400 mM) and BQ788 (1 μM); and the pulmonary vascular response was compared between Groups B1 and B2.

Experimental protocol

During the baseline period, lungs were ventilated with room air under constant perfusion flow (10 mL/min). Subsequently, the preparations were randomized into four groups (A1, $n = 8$; A2, $n = 8$; B1, $n = 8$; and B2, $n = 8$) and sequentially challenged by graded increase in perfusion flow from 10 mL/min, to 13 mL/min, 18 mL/min, and 25 mL/min. In Groups A2 and B2, the isolated lungs were pretreated with L-NAME 15 minutes before the flow challenge and 5 minutes later, with BQ788, 10 minutes before commencing the flow challenge. Following each challenge of flow alteration, the pH, gas tension in the perfusate, and PAP were obtained after steady PAP values were observed over a period of at least 10 minutes. Thereafter the inspired gas was switched to the following mixture: (1) Groups A1 and A2: normoxic-hypercapnia gas with 5% CO_2 in air and (2) Groups B1 and B2: hypoxic-hypercapnia gas with 5% CO_2 in N_2. After 10 minutes of experimental gas inhalation, the changes of PAP, pH, and the gas tension in the perfusate were recorded. The inspired gas was then switched back to room air for 10 minutes before the next challenge of flow alteration and gas inhalation.

Statistical analysis

Values are expressed as means ± standard deviation or means ± standard error of the mean as appropriate. Statistical evaluation of the differences among and within groups was performed using the paired Student *t* test. The *p* values less than 0.05 were considered to be statistically significant.

Results

CO_2 on the flow-associated PAP elevation under normoxic-hypercapnia ventilation with and without endogenous NO

In Experiment Series A, ventilation with normoxic-hypercapnia gas produced a significant increase in $PaCO_2$ ($p < 0.01$) and a decrease in pH ($p < 0.01$) (Table 1). Three challenges (10–13 mL/min, 18 mL/min, and 25 mL/min) of increased perfusion flow elevated the PAP by 4.0 ± 1.0 mmHg, 6.5 ± 1.3 mmHg, and 7.6 ± 1.2 mmHg by a significant amount ($p < 0.01$) (Table 1, Fig. 1A). Neither inhalation of normoxic-hypercapnia nor normal room air breathing had a significant influence on the PAP (Fig. 1A and B).

In Group A2, there was no significant change on basal PAP with L-NAME and BQ788 pretreatment. In this group, we observed that the flow-associated PAP elevation was not affected under endogenous NO inhibition ($p > 0.05$, compared with Group A1) (Fig. 3). The increases in PAP were 4.4 ± 1.1 mmHg, 7.1 ± 0.9 mmHg, and 8.0 ± 0.5 mmHg by three sequential flow alterations ($p < 0.01$) (Table 1, Fig. 1A). Neither normoxic-hypercapnia gas inhalation nor room air breathing changed the PAP significantly (Fig. 1A and C).

CO_2 on the flow-associated PAP elevation under hypoxic-hypercapnia ventilation with and without endogenous NO

In Experiment Series B, ventilation with hypoxic-hypercapnia gas produced a significant increase in $PaCO_2$

Table 1 pH, PCO_2 in the perfusate, and PAP during the different experimental conditions

Treatment:	Baseline (10 lpm)	13 lpm	Gas inhalation	Room air	18 lpm	Gas inhalation	Room air	25 lpm	Gas inhalation	Room air
Group A1										
PAP	14.4 ± 0.9	18.4 ± 0.9	18.2 ± 0.8	18.2 ± 0.7	24.7 ± 1.4	24.6 ± 1.2	24.8 ± 1.1	32.4 ± 2.1	31.6 ± 2.0	32.0 ± 2.1
PCO_2		34.6 ± 1.4	65.2 ± 5.2[a]		36.4 ± 2.4	68.8 ± 6.6[a]		37.5 ± 2.8	64.7 ± 6.1[a]	
pH		7.34 ± 0.08	7.20 ± 0.10[a]		7.35 ± 0.06	7.14 ± 0.09[a]		7.39 ± 0.08	7.13 ± 0.11[a]	
Group A2										
PAP	14.7 ± 1.3	19.0 ± 1.2	19.1 ± 1.4	19.1 ± 1.0	26.2 ± 1.3	26.0 ± 1.4	25.8 ± 1.3	33.8 ± 1.5	33.3 ± 1.7	33.3 ± 1.6
PCO_2		38.9 ± 0.6	72.8 ± 6.4[a]		39.9 ± 0.8	68.7 ± 5.9[a]		42.1 ± 1.8	72.2 ± 6.6[a]	
pH		7.39 ± 0.06	7.08 ± 0.12[a]		7.34 ± 0.9	7.19 ± 0.10[a]		7.43 ± 0.16	7.05 ± 0.15[a]	
Group B1										
PAP	15.0 ± 1.0	19.1 ± 1.2	21.1 ± 1.3	20.2 ± 1.2	25.6 ± 1.3	28.7 ± 1.5	27.0 ± 1.2	34.1 ± 1.2	38.2 ± 1.9	35.4 ± 1.8
			→18.6 ± 1.2			→25.1 ± 1.2			→33.1 ± 1.5	
PCO_2		34.6 ± 0.8	68.2 ± 4.9[a]		39.2 ± 1.3	65.6 ± 5.9[a]		41.2 ± 1.7	64.2 ± 5.3[a]	
pH		7.34 ± 0.04	7.18 ± 0.09[a]		7.38 ± 0.05	7.15 ± 0.09[a]		7.38 ± 0.08	7.14 ± 0.14[a]	
Group B2										
PAP	14.6 ± 1.4	18.7 ± 1.4	21.9 ± 1.7	20.7 ± 1.5	26.9 ± 0.9	32.8 ± 1.3	28.9 ± 1.1	36.8 ± 2.1	45.2 ± 1.78	38.5 ± 2.2
			→18.6 ± 1.5			→26.9 ± 1.5			→35.8 ± 2.1	
PCO_2		39.2 ± 0.7	67.8 ± 6.6[a]		36.2 ± 0.9	63.2 ± 5.9[a]		34.5 ± 0.4	63.2 ± 6.4[a]	
pH		7.40 ± 0.08	7.11 ± 0.13[a]		7.35 ± 0.09	7.17 ± 0.11[a]		7.33 ± 0.07	7.09 ± 0.14[a]	

[a] Less than 0.01 compared with corresponding values before gas inhalation.
Gas inhalation: Group A1 = 5% CO_2 in air; Group A2 = 5% CO_2 in air, pretreated with L-NAME + BQ788; Group B1 = 5% CO_2 in N_2; Group B2 = 5% CO_2 in N_2, pretreated with L-NAME + BQ788; PAP = pulmonary arterial pressure (mmHg).

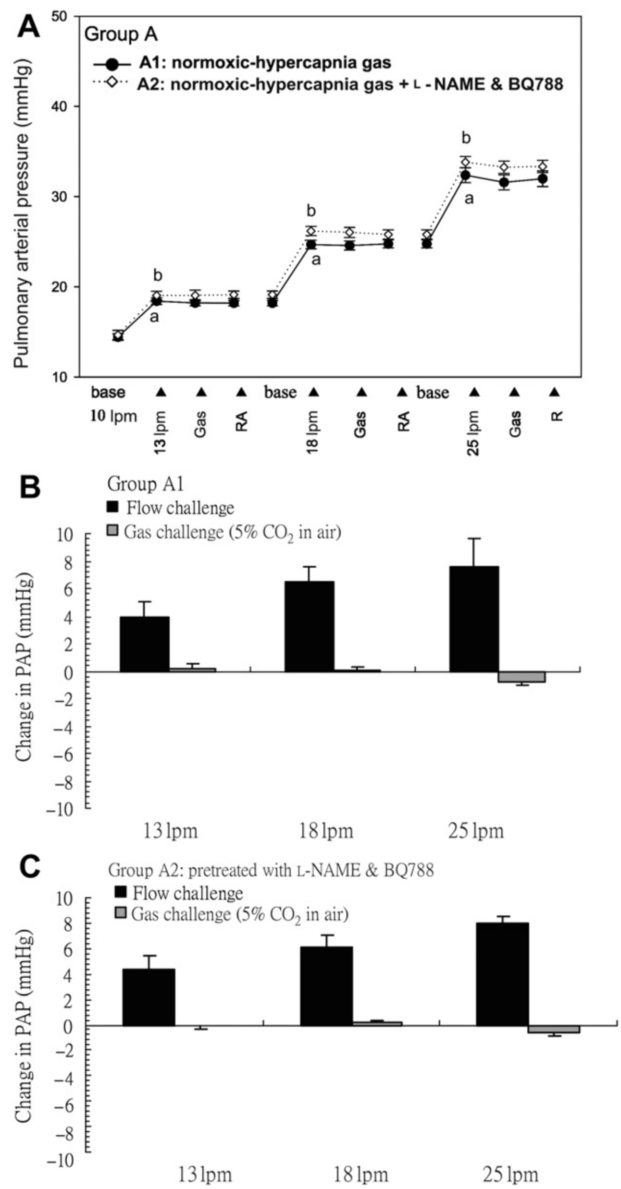

Figure 1. (A) Graph representing means ± standard error, PAP at baseline, and during the course of the experiment in Series A. PAP increased significantly in response to flow alternation ($^{a,b}p < 0.01$ compared with base). (B) and (C) Values are means ± standard deviation. PAP changes in response to normoxic-hypercapnia gas (5% CO_2 in air) following with flow challenges at various speeds for Groups A1 and A2. Normoxic-hypercapnia gas (5% CO_2 in air) has no influence on PAP. CO_2 = carbon dioxide; L-NAME = *N*-nitro-L-arginine methyl ester; PAP = pulmonary arterial pressure; RA = room air.

($p < 0.01$) and a decrease in pH ($p < 0.01$) (Table 1). In Group B1, the perfusion flow at rates of 13 mL/min, 18 mL/min, and 25 mL/min elevated the PAP by 4.1 ± 0.7 mmHg, 4.7 ± 1.0 mmHg, and 7.1 ± 0.9 mmHg, respectively ($p < 0.01$) (Table 1, Fig. 2A). Hypoxic-hypercapnia ventilation (5% CO_2 in N_2) evoked a biphasic response with transient hypoxic vasoconstriction. PAP was initially increased by 2.0 ± 0.4 mmHg, 3.1 ± 0.7 mmHg, and 4.1 ± 0.9 mmHg ($p < 0.05$). However, after 4—6 minutes of gas inhalation, PAP began to gradually drop; after 10 minutes of gas inhalation, PAP had decreased by 2.4 ± 0.5 mmHg, 3.6 ± 0.6 mmHg, and 5.2 ± 0.9 mmHg ($p < 0.01$) (Table 1, Fig. 2A and B). The PAP rebounded by 1.5 ± 0.3 mmHg, 2.0 ± 0.3 mmHg, and 2.4 ± 0.4 mmHg, respectively, when the inhaled gas was changed to room air (Fig. 2A). In Group B2, inhibition of NO synthesis with L-NAME and BQ788 also evoked a biphasic response with a transient hypoxic vasoconstriction. In this group, the PAP was elevated by 4.1 ± 0.7 mmHg, 5.4 ± 0.9 mmHg, and 8.0 ± 2.0 mmHg during three challenges of flow alteration ($p < 0.01$) (Table 1, Fig. 2A). In response to hypoxic-hypercapnia gas (5% CO_2 + N_2), PAP was initially increased by 3.3 ± 0.6 mmHg, 5.9 ± 0.9 mmHg, and 8.3 ± 1.7 mmHg ($p < 0.01$). At 4—6 minutes of gas inhalation, PAP began to gradually drop; at 10 minutes of gas inhalation, PAP had decreased by 3.4 ± 0.8 mmHg, 5.9 ± 1.0 mmHg, and 9.4 ± 1.6 mmHg ($p < 0.01$) (Table 1, Fig. 2A and C). Again, room air inhalation reversed the PAP by 2.2 ± 0.7 mmHg, 2.0 ± 0.5 mmHg, and 2.7 ± 0.4 mmHg (Fig. 2A). In this series of experiments, pretreatment with L-NAME and BQ788 eliminated the endogenous NO, while greatly potentiating the pulmonary vasoconstriction response to hypoxia, but did not eliminate the vasodilatory effect of CO_2 (Fig. 2C).

Discussion

In the present study, we demonstrated that CO_2 provides a compensatory mechanism to reverse the PAP when vascular tone is raised by hypoxia, but not by alternation of perfusion flow (Figs. 1A and 2A). Pretreatment with L-NAME and BQ788 significantly potentiates hypoxic pulmonary vasoconstriction (Fig. 2A). However, the pulmonary vasodilatory effects of CO_2 are essentially not affected by L-NAME or BQ788 (Fig. 2D), suggesting that NO is not involved in the hypercapnic vasodilatation.

There is strong evidence that pulmonary vessels constrict during hypoxia, hypercapnia, and acidemia. High CO_2 tension with elevated hydrogen ion concentration in the blood increases the extracellular Ca^{2+} influx, which is thought to be the main cause of vasoconstriction in the pulmonary circulation. The early works of Duke et al. [29] and Shaw and Barer [8] showed that CO_2 usually causes weak vasoconstriction under normal vascular tone. Addition of acid also causes vasoconstriction, whereas alkali administration causes vasodilation. However, CO_2 also plays a vasodilator role under the condition of high vascular tone. Subsequent studies have reported that respiratory acidosis tends to attenuate the suppressor response to hypoxia and vasoconstrictors, whereas respiratory alkalosis exerts the opposite effect [9,10]. These findings suggest that an increase in hydrogen ion concentration alone causes pulmonary vasoconstriction and that an increase in CO_2 tension in the blood could attenuate the vasomotor response to hypoxia or vasoconstrictors in spite of the hydrogen ion concentration. These findings are consistent with those of Viles and Shepherd [7], who showed CO_2 acted as a pulmonary vasodilator, independent of hydrogen ion concentration. In this connection, our previous study also confirmed that the vasodilatory effect of CO_2 is pH independent. In the experiment, under ET-1—induced

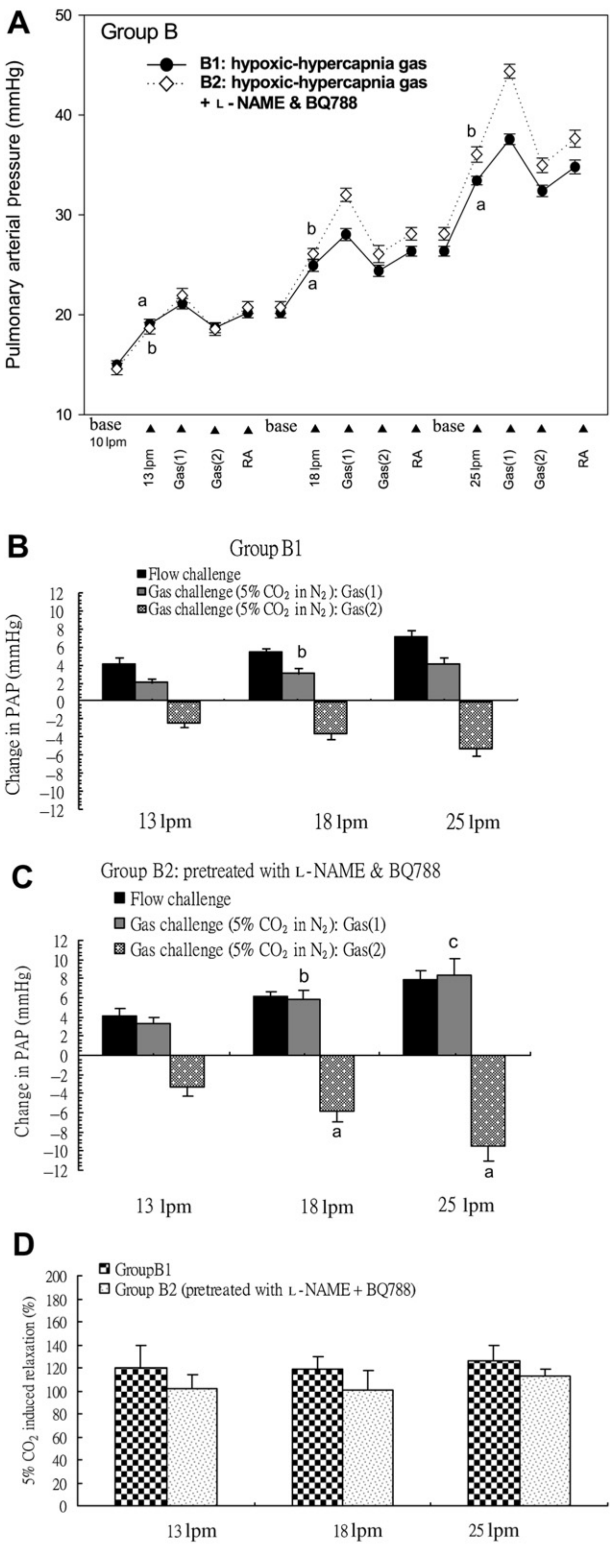

Figure 2. (A) Graph representing means $\pm$ standard error, PAP at baseline, and during the course of the experiment in Series B. PAP increased significantly in response to speed alternation ($^{a,b}p < 0.01$ compared with base). In both groups, hypoxic-hypercapnia gas challenge (5% CO_2 in N_2) evoked a biphasic response with a transient hypoxic vasoconstriction [first phase, Gas(1)] followed by a CO_2 vasodilatation [second phase, Gas(2)]. (B) and (C) PAP changes in response to hypoxic-hypercapnia gas (5% CO_2 in N_2) following with flow challenges at various speeds for Groups B1 and B2. (Values are means $\pm$ standard deviation. $^{c}p < 0.05$; $^{b}p < 0.01$ hypoxic vasoconstriction compared with previous course of gas challenge. $^{a}p < 0.01$ CO_2 vasodilatation *vs.* previous course of gas challenge). (D) Values are means $\pm$ standard deviation. Percent relaxation in response to CO_2 showed no significant difference between Groups B1 and B2. The vasodilatation effect of CO_2 was not affected by L-NAME and BQ788. CO_2 = carbon dioxide; L-NAME = *N*-nitro-L-arginine methyl ester; PAP = pulmonary arterial pressure; RA = room air.

pulmonary hypertension, we infused acetic acid to alter the pH value close to the value produced by 5% CO_2 inhalation. The infused acetic acid slightly elevated the PAP while decreasing the pH [20].

The action of CO_2 on vascular tone was described to be a local action because it was present after autonomic blockade in isolated perfused lungs and was not eliminated in intact animals by vagotomy or atropine [11]. There has been considerable interest in the role of NO in mediating hypercapnic vasodilatation. In the present study, blocking endogenous NO with L-NAME and BQ788 did not eliminate the vasodilatory response to hypercapnia, but enhanced hypoxic pulmonary vasoconstriction. NO seems to specifically modulate hypoxic pulmonary vasoconstriction while not being involved in CO_2-induced vasodilation. Several studies have pointed out that an increase in NO production during acute or chronic hypoxia tends to blunt the vasoconstrictory effect induced by hypoxia [11,30—33]. In the present data, in Group B1 we observed a biphasic response of transient hypoxic vasoconstriction followed by CO_2-induced vasodilatation in response to hypoxic-hypercapnia (Fig. 2A and B). In Group B2, inhibition of endogenous NO tended to potentiate the pulmonary vasoconstriction response to hypoxia, but did not eliminate the vasodilatory effect of CO_2. In both the B1 and B2 groups, this latter pulmonary vasodilatation could be aborted with pure N2 inhalation (data not shown). These findings suggest that acute hypoxia causes pulmonary vasoconstriction, but coexistent hypercapnia inhibits this effect. Comparing the percentages of CO_2-induced vasodilatation in both groups, the vasodilatory effect of CO_2 was not affected by inhibition of endogenous NO (Fig. 2D). This suggests that NO is significantly involved in hypoxic vasoconstriction, whereas not contributing to hypercapnic ventilation in the face of hypoxic pulmonary hypertension. In contrast to our finding, Yamaguchi et al. [34] documented that hypercapnic acidosis elevated vascular tone and perfusate nitrite/nitrate in an isolated lung model. Other studies have also reported that hypercapnia acidosis is associated with the upregulation of nitric oxide synthase-mediated NO dependent effects at the vascular and molecular levels [35,36]. Although our results differ from previous studies, it appears that acidification may stimulate unidentified mechanisms in the pretranscriptional phase of endothelial nitric oxide synthase [37,38].

Study evidence suggests that normal pulmonary vascular tone is regulated by vasoactive substances that are produced locally by the vascular endothelium. The phenomenon of enhanced basal NO production also occurs in certain types of pulmonary hypertension that is an important modulator of pulmonary vascular reactivity [24,26]. However, the response of the pulmonary vasculature to increase in blood flow appears to be very contradictory. Some reports indicate that increased blood flow produces endothelial dysfunction with loss of endothelial dependent vasodilatation [27–39]. In the present study, we did not observe the phenomenon of flow-associated NO enhancement in comparing the flow-induced PAP elevation among the groups with and without inhibition of endogenous NO (Fig. 3). Furthermore, from the observation in the study of Series B, the hypoxic vasoconstriction response was potentiated with inhibition of endogenous NO. If NO formation was deprived in flow-induced pulmonary hypertension, endogenous NO inhibition should not cause a higher magnitude of pressure rise in response to hypoxia stimulation.

As mentioned in the literature review, there is no significant difference in pulmonary vascular resistance in response to graded CO_2 or varying concentrations of CO_2 [8,9,34,40]. In the present study, we simply used a concentration of 5% CO_2, which produced a degree of hypercapnia acidosis similar to that commonly observed when using protective ventilatory strategies in clinical illness. Reports to date of the vasoactive action of CO_2 have concentrated on its vasodilatory and beneficial effects. Although we know that discrepant vasoactive action of CO_2 may arise from differences in pulmonary vascular tone, the concept of a pressure-response relationship between the degree of CO_2-induced vasodilatation and the level of PAP still needs to be elucidated. Therefore, our study was in large part designed to identify whether the vasodilator effect of CO_2 on the pulmonary circulation is dependent on the level of PAP and to assess the effect of CO_2 on pulmonary vascular tone under various conditions. In clinical situations, hypercapnia, hypoxia, and increased blood flow usually coexist in pulmonary hypertension. There has been considerable interest in the effect of CO_2 on hypoxic and flow alteration–induced pulmonary hypertension. However, in the present study, we observed that CO_2 reversed the PAP only when induced by hypoxia stimulation, but not when induced by alteration of perfusion flow. Pulmonary hypertension secondary to increased pulmonary blood flow and hypoxic

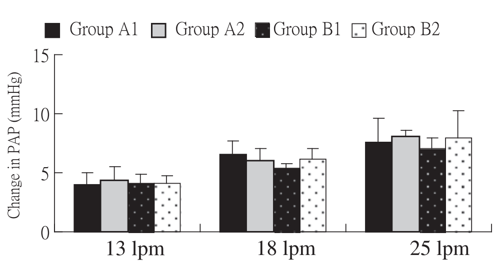

Figure 3. Graph representing means ± standard deviation increase in pulmonary arterial pressure at varying perfusion flow rate (13 mL/min, 18 mL/min, and 25 mL/min) in all study groups. There are no significant differences between groups in response to each challenge of flow alternation ($p > 0.05$). Group A1: 5% carbon dioxide (CO_2) in air; Group A2: 5% CO_2 in air, pretreated with *N*-nitro-L-arginine methyl ester + BQ788; Group B1: 5% CO_2 in N_2; Group B2: 5% CO_2 in N_2, pretreated with *N*-nitro-L-arginine methyl ester + BQ788. PAP = pulmonary arterial pressure.

pulmonary vasoconstriction often coexists with altered vascular reactivity. The discrepant results may arise from the specific vasodilatory action of CO_2. The vasoactive action of CO_2 did not affect the flow-associated pulmonary hypertension because the pulmonary vessels act as a distensible tube that enlarges with increasing flow, leading to increased PAP.

The major findings of the present study are encouraging in that elevated CO_2 tension in arterial blood might have a protective effect and this could have important implications for the clinical management of mechanical ventilation in intensive care settings. In conclusion, there are several aspects of our present study that provide evidence that (1) inhaled CO_2 reversed pulmonary hypertension induced by hypoxia but not by flow alteration; (2) the vasodilatory effects of CO_2 at different pressure levels vary in accordance with the levels of PAP—the dilatory effect tends to be more evident at higher PAP; and (3) endogenous NO attenuates hypoxic pulmonary vasoconstriction but does not augment the CO_2-induced vasodilatation.

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References

[1] Schreiber MD, Heymann MA, Soifer SJ. Increased arterial pH, not decreased $PaCO_2$ attenuates hypoxia-induced pulmonary vasoconstriction in newborn lambs. Pediatr Res 1986;20: 112–7.

[2] Hammer J. Acute lung injury: pathophysiology, assessment and current therapy. Paediatr Respir Rev 2001;2:10–21.

[3] Laffey JG, O'Croinin D, McLoughlin P, Kavanagh BP. Permissive hypercapnia—role in protective lung ventilatory strategies. Intensive Care Med 2004;30:347–56.

[4] Temmesfeld-Wollbrück B, Walmrath D, Grimminger C, Seeger W. Prevention and therapy of the adult respiratory distress syndrome. Lung 1995;173:139–64.

[5] Brimioulle S, Lejeune P, Vachiery JL, Leeman M, Melot C, Naeije R. Effects of acidosis and alkalosis on hypoxic pulmonary vasoconstriction in dogs. Am J Physiol 1990;258: 347–53.

[6] Cutaia M, Rounds S. Hypoxic pulmonary vasoconstriction: physiologic significance, mechanism and clinical relevance. Chest 1990;97:706–18.

[7] Viles PH, Shepherd JT. Relationship between pH, PO_2, and PCO_2 on the pulmonary vascular bed of the cat. Am J Physiol 1968;215:1170–6.

[8] Shaw JW, Barer GR. Pulmonary vasodilator and vasoconstrictor actions of carbon dioxide. J Physiol 1971;213:633–45.

[9] Schreiber MD, Soifer SJ. Respiratory alkalosis attenuates thromboxane-induced pulmonary hypertension. Crit Care Med 1998;16:1225–8.

[10] Malik AB, Kidd BS. Independent effects of change in H^+ and CO_2 concentrations on hypoxic pulmonary vasoconstriction. J Appl Physiol 1973;34:318–23.

[11] Benumof JL, Wahrenbrock EA. Blunted hypoxic pulmonary vasoconstriction by increased lung vascular pressures. J Appl Physiol 1975;38:846–50.

[12] Laffey JG, Tanaka M, Engelberts D, Luo X, Yuan S, Keith TA, et al. Therapeutic hypercapnia reduces pulmonary and systemic injury following *in vivo* lung reperfusion. Am J Respir Crit Care Med 2000;162:2287—94.
[13] Broccard AF, Hotchkiss JR, Vannay C, Markert M, Sauty A, Feihl F, et al. Protective effect of hypercapnic acidosis on ventilator-induced lung injury. Am J Respir Crit Care Med 2001;164:802—6.
[14] Laffey JG, Honan D, Hopkins N, Hyvelin JM, Boylan JF, McLoughlin P. Hypercapnic acidosis attenuates endotoxin-induced acute injury. Am J Respir Crit Care Med 2004;169: 46—56.
[15] Kregenow DA, Rubenfeld GD, Hudson LD, Swenson ER. Hypercapnic acidosis and mortality in acute lung injury. Crit Care Med 2003;4:1—7.
[16] Chonghaile M, Higgins BD, Costello JF, Laffey JG. Hypercapnic acidosis attenuates severe acute bacterial pneumonia-induced lung injury by a neutrophil-independent mechanism. Crit Care Med 2008;36:3135—44.
[17] Kregenow DA, Swenson ER. The lung and carbon dioxide: implications for permissive and therapeutic hypercapnia. Eur Respir J 2002;20:6—11.
[18] Kantores C, McNamara PJ, Teixeira L, Engelberts D, Murthy P, Kavanagh BP, et al. Therapeutic hypercapnia prevents chronic hypoxia-induced pulmonary hypertension in the newborn rat. Am J Physiol Lung Cell Mol Physiol 2006;291:912—22.
[19] Sinclair SE, Kregenow DA, Starr IR, Chi EY, Schimmel C, Lamm JE, et al. Therapeutic hypercapnia and ventilation-perfusion matching in acute lung injury: low minute ventilation *vs* inspired CO_2. Chest 2006;130:85—92.
[20] Chuang IC, Dong HP, Yang RC, Wang TH, Tsai JH, Yang PH, et al. Effect of carbon dioxide on pulmonary vascular tone at various pulmonary arterial pressure levels induced by endothelin-1. Lung 2010;188:199—207.
[21] Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987;327:524—6.
[22] Yanagisawa M, Kurihare H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;323:411—5.
[23] Markewitz BA, Kohan DE, Michael JR. Endothelin-1 synthesis, receptors and signal transduction in alveolar epithelium: evidence for an autocrine role. Am J Physiol 1995;268:192—200.
[24] Dai ZK, Tan MS, Chai CY, Chen IJ, Jeng AY, Wu JR. Effects of increased pulmonary flow on the expression of endothelial nitric oxide synthase and endothelin-1 in the rat. Clin Sci 2001;103:289s—93s.
[25] Sharma S, Kumar S, Sud N, Wiseman DA, Tian J, Rehmani I, et al. Alteration in lung arginine metabolism in lambs with pulmonary hypertension associated with increased pulmonary blood flow. Vasc Pharmacol 2009;51:359—64.
[26] Chou TF, Wu MS, Chien CU, Yu CC, Chen CF. Enhanced expression of nitric oxide synthase in the early stage after increased pulmonary blood flow in rats. Eur J Cardiothoracic Surg 2002;21:331—6.
[27] Chen HI, Hu CT, Wu CY, Wang D. Nitric oxide in systemic and pulmonary hypertension. J Biomed Sci 1997;4:244—8.
[28] Baker DG, Toth BR, Goad MEP, Barker SA, Means JC. Establishment and validation of an isolated rat lung model for pulmonary metabolism studies. J Appl Toxicol 1999;19: 83—91.
[29] Duke HN, Killick EM, Marchant JV. Changes in pH of the perfusate during hypoxia in isolated perfused cat lungs. J Physiol 1960;153:413—22.
[30] Liu SF, Crawley DE, Barnes PJ, Evans TW. Endothelium-derived relaxing factor inhibits hypoxic pulmonary vasoconstriction in rats. Am Rev Respir Dis 1991;143:32—7.
[31] Deleuze PH, Shiiya AN, Thoraval R, Eddahibi S, Braquet P, Chabrier PE, et al. Endothelin dilates bovine pulmonary circulation and reverses hypoxic pulmonary vasoconstriction. J Cardiovas Pharmacol 1992;19:354—60.
[32] Dorrington KL, Talbot NP. Human pulmonary vascular responses to hypoxia and hypercapnia. Pflűgers Arch 2004; 449:1—15.
[33] Hampl V, Archer SL, Nelson DP. Chronic EDRF inhibition and hypoxia: effects on pulmonary circulation and systemic blood pressure. J Appl Physiol 1993;75:1748—57.
[34] Yamaguchi K, Takasugi T, Fujuta H, Mori M, Suzuk Y. Endothelial modulation of pH-dependent presser response in isolated perfused rabbit lungs. Am J Physiol 1996;270:252—8.
[35] Pedoto A, Caruso JE, Nandi J, Oler A, Hoffman SP, Tassiopoulos AK, et al. Acidosis stimulates nitric oxide production and lung damage in rats. Am J Respir Crit Care Med 1999;159:397—402.
[36] Serrano Jr CV, Fraticelli A, Paniccia R, Teti A, Noble B, Corda S, et al. PH dependence of neutrophil-endothelial cell adhesion and adhesion molecule express. Am J Physiol 1996; 271:962—70.
[37] Mizuno S, Demura Y, Ameshima S, Okamura S, Miyamori I, Ishizaki T. Alkalosis stimulates endothelial nitric oxide synthase in cultured human pulmonary arterial endothelial cells. Am J Physiol Lung Cell Mol Physiol 2002;283:113—9.
[38] Najarian T, Marrache AM, Dumont I, Hardy P, Beauchamp MH, Hou X, et al. Prolonged hypercapnia-evoked cerebral hyperemia via K^+ channel-and prostaglandin E2-dependent endothelial nitric oxide synthase induction. Circ Res 2000;87: 1149—56.
[39] Lockette W, Ostuka Y, Carretero O. The loss of endothelium-dependent relaxation in hypertension. Hypertension 1986;8: II61—6.
[40] Barer GR, Howard P, Shaw JW. Sensitivity of pulmonary vessels to hypoxia and hypercapnia. J Physiol 1970;206:25—6.