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In vivo support for the new concept of pulmonary blood flow-mediated CO₂ gas excretion in the lungs

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1Department of Physiology, Shinshu University School of Medicine, Matsumoto, Japan; 2Department of Innovation of Medical and Health Sciences Research, Shinshu University School of Medicine, Matsumoto, Japan; 3Department of Anesthesiology and Resuscitology, Shinshu University School of Medicine, Matsumoto, Japan; and 4Hitachi Aloka Medical, Tokyo, Japan

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Kawai Y, Ajima K, Kaidoh M, Sakaguchi M, Tanaka S, Kawamata M, Kimura H, Ohhashi T. In vivo support for the new concept of pulmonary blood flow-mediated CO₂ gas excretion in the lungs. Am J Physiol Lung Cell Mol Physiol 308: L1224–L1236, 2015. First published April 17, 2015; doi:10.1152/ajplung.00205.2014.—To further examine the validity of the proposed concept of pulmonary blood flow-dependent CO₂ gas excretion in the lungs, we investigated the effects of intramediastinal balloon catheterization-, pulmonary artery catheterization-, or isoprenaline (ISP)-induced changes in pulmonary blood flow on the end-expiratory CO₂ gas pressure (P̄ECO₂), the maximal velocity of the pulmonary artery (Max Vp), systemic arterial pressure, and heart rate of anesthetized rabbits. We also evaluated the changes in the P̄ECO₂, in clinical models of anemia or pulmonary embolism. An almost linear relationship was detected between the P̄ECO₂ and Max Vp. In an experiment in which small pulmonary arteries were subjected to stenosis, the P̄ECO₂ fell rapidly, and the speed of the reduction was dependent on the degree of stenosis. ISP produced significant increases in the P̄ECO₂ of the anesthetized rabbits. Conversely, treatment with piceatannol or acetazolamide induced significant reductions in the P̄ECO₂. Treatment with a cell surface F₁/F₀ ATP synthase antibody caused significant reductions in the P̄ECO₂ itself and the ISP-induced increase in the P̄ECO₂. Neither the P̄ECO₂ nor SAP was significantly influenced by marked anemia [%hematocrit (Ht), 70–47%]. On the other hand, in the presence of less severe anemia (%Ht: 100–70%) both the P̄ECO₂ and SAP fell significantly when the rabbits’ blood viscosity was decreased. The rabbits in which pulmonary embolisms were induced demonstrated significantly reduced P̄ECO₂ values, which was compatible with the lowering of their Max Vp. In conclusion, we reaffirm the validity of the proposed concept of CO₂ gas exchange in the lungs.

VASCULAR ENDOTHELIAL CELLS (EC) are constantly exposed to shear stress, the mechanical force generated by blood flow. The shear stress in a Newtonian fluid is defined as follows: shear stress = viscosity × flow velocity gradient (dv/dt), so if the viscosity is constant the level of shear stress depends upon the flow velocity gradient (23). EC recognize shear stress and transmit signals to their interior, where they trigger cellular responses, including changes in a variety of cell functions (3, 5, 12, 25). For example, in response to shear stress, vascular EC release endogenous ATP, which is produced by the activation of cell surface F₁/F₀ ATP synthase (25). Yamamoto et al. (25) also demonstrated that pulmonary arterial EC exhibited the most prominent responses to shear stress. However, how ATP release from pulmonary arteriolar EC, which might be exposed to higher shear stress levels than the EC in the pulmonary artery, is affected by shear stress is unclear. The following continuity equation suggests that the pulmonary blood flow volume-dependent flow velocity gradient of the pulmonary arterioles will be greater than that of the pulmonary artery providing the cross-sectional area of the blood vessels remains constant: (the blood flow volume = the cross-sectional area of the blood vessel × the mean flow velocity) (Fig. 1). Therefore, the pulmonary blood flow-dependent changes in shear stress that occur in the pulmonary arterioles are expected to be greater than those seen in the pulmonary artery. It should also be noted that no previous studies have evaluated the physiological functions/effects of the H⁺ that is coreleased from pulmonary arteriolar EC during the activation of cell surface F₁/F₀ ATP synthase.

Recently, we (13) demonstrated that 10 s shear stress stimulation induced stress strength-dependent H⁺ release followed by CO₂ gas excretion from pulmonary arteriolar EC, which was significantly abrogated by the inhibition of F₁/F₀ ATP synthase or carbonic anhydrase (CA) type IV on the EC surface. Based on these findings, we proposed a new concept of CO₂ gas excretion in the human lungs (Fig. 1), pulmonary arteriolar flow-mediated cell surface F₁/F₀ ATP synthase-dependent H⁺ secretion, which results in the facilitation of a dehydration reaction involving HCO₃⁻ in the plasma followed by CO₂ gas excretion from arteriolar EC. Based on this concept, we expect that pulmonary blood flow velocity will have a greater effect on the changes in shear stress that occur in the pulmonary arterioles than those seen in the pulmonary artery.

On the other hand, the classical concept of CO₂ gas exchange in the lungs is well established (14). According to this concept, the first step in CO₂ gas exchange involves a combination of the physiological processes required for CO₂ gas diffusion from cells in tissues, the catalysis of carbonic acid
Proposed new concept of pulmonary blood flow-mediated CO₂ gas excretion in the lungs.

![Diagram of CO₂ gas exchange in pulmonary arterioles]

**Fig. 1.** Schematic diagram of our proposed new concept of CO₂ gas excretion in the human lungs. Pulmonary arteriolar flow-mediated cell surface F₁/F₀ ATP synthase-dependent H⁺ secretion results in the carbonic anhydrase (CA) IV-induced facilitation of a dehydration reaction involving HCO₃⁻ in the plasma, followed by CO₂ gas excretion from pulmonary arteriolar endothelial cells (EC). According to the continuity equation mentioned in the text, the pulmonary blood flow-dependent changes in the flow velocity gradient (shear stress) should be greater in pulmonary arterioles than in pulmonary arteries, provided that the cross-sectional area of the blood vessels does not change significantly. In other words, variations in pulmonary blood flow resulted in greater changes in shear stress in pulmonary arterioles than in pulmonary arteries.

**CO₂ GAS EXCHANGE IN PULMONARY ARTERIOLES**

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### MATERIALS AND METHODS

**In Vivo Animal Experiments**

**Animals, anesthesia, and monitoring.** This study was approved by the Institutional Animal Care and Use Committee of Shinshu University. Forty-one 22- to 26-mo-old male Japanese white rabbits weighing 3.0–3.5 kg (3.2 ± 0.1 kg, n = 41) were used in the animal experiments in a humane and ethical fashion. The animals were fasted overnight, and preanesthesia sedation was induced via the intramuscular administration of 0.1–0.2 mg/kg medetomidine hydrochloride (Orion Pharma, Helsinki, Finland) and 0.7–1.2 mg/kg midazolam (Astellas Pharma, Tokyo, Japan). Anesthesia was maintained with 2.0–3.0% isoflurane [under N₂O + 100% O₂ inhalation (2:1 N₂O-O₂; Dainippon Sumitomo Pharma, Tokyo, Japan), titrated to effect after the lower part of the thyroid gland had been subjected to a tracheostomy. Ventilation was maintained at 40 ml/breath for 20 breaths/min to minimize the effects of ventilation-perfusion mismatch on the rabbits’ PECO₂.

In some experiments, to examine the effects of a smaller tidal volume or the administration of 100% FIO₂ on the pulmonary blood flow-dependent responses of the PECO₂, the ventilation parameters were changed to 20 ml/breath and 30 breaths/min, or from N₂O + 100% O₂ (PECO₂) to 100% O₂ (100% FIO₂). During these experiments, we measured the arterial partial pressures of O₂ and CO₂ (Paco₂), and arterial pH using a blood gas analyzer (ABL5; Radiometer, Copenhagen, Denmark).

Any changes in CO₂ pressure that occurred during ventilation were recorded using a CO₂ monitor and a CO₂ sensor kit (OLG-2800 and TG970P; Nihon Kohden, Tokyo, Japan) connected to a transesophageal cannula (Fig. 2, A and B). SAP was monitored with a pressure transducer (MOD-002, Skinos; Sakaki, Nagano, Japan) attached to a catheter that had been inserted in the femoral artery. Physiological saline solution (PSS; Otsuka Pharma, Tokyo, Japan) was administered at a rate of 18 ml/h during the experiments (Fig. 2A). The animals were killed after the experiments.

**Echocardiography procedure.** Conventional transcutaneous, contrast-specific gray-scale, or color-flow Doppler echocardiography was performed by a professional ultrasound operator using a EUB-7500 ultrasound scanner (Hitachi, Tokyo, Japan) and a 7.5-MHz pediatric cardiac transducer (EUP-S52; Hitachi) over the third or fourth intercostal space at the left margin of the sternum (Fig. 2A). The Max Vp, pulmonary artery diameter, and HR of the anesthetized rabbits were measured using echocardiography and the associated computer system (Fig. 2C).

**Experimental procedure and protocols.** The rabbits were placed on the operating table in the supine position. The body temperature of each animal was maintained at 36.5–37.5°C using a heating pad.
To examine the effects of pulmonary arterial blood flow on the PECO2 of the anesthetized rabbits, the thorax was opened by cutting along the left margin of the sternum, and a balloon catheter was inserted in the mediastinal cavity and fixed to the pericardium just over the cardiac outlet of the pulmonary artery (Fig. 2A). Pulmonary blood flow was then altered by expanding the balloon. In addition, in some experiments, to selectively decrease blood flow through the smaller pulmonary arteries or arterioles a pulmonary artery catheter (Swan-Ganz thermodilution Catheter, 132F; Edwards Lifesciences, Irvine, CA) was inserted in the target blood vessel via the middle portion of the external jugular vein. After each change in pulmonary blood flow, the rabbits’ PECO2, Max Vp, SAP, and HR were recorded. During each experiment, we measured the mixed venous PCO2 of the blood delivered from the pulmonary artery using the blood gas analyzer to confirm that it did not vary markedly between the experiments.

To evaluate the effects of drug-mediated changes in pulmonary blood flow on the PECO2, Max Vp, SAP, and HR of the anesthetized rabbits, a single dose of ISP (0.31 μg/kg; Kowa, Tokyo, Japan) was administered in the femoral vein in the presence or absence of an end-expiratory CO2 gas pressure (mmHg). A representative PECO2 recording is shown in B. A1: closed lung. Closed lung preparations were used for the experiments evaluating the effects of: 1) changes in the hematocrit (Ht) value, 2) the administration of a single iv injection of the cell surface F1/FO ATP synthase inhibitor piceatannol or the CA IV inhibitor acetazolamide (ACZ), 3) the administration of a single iv injection of isoprenaline (ISP) in the presence or absence of phentolamine and/or propranolol, or 4) lung emboli produced by the administration of a single iv injection of the collagen-based embolic agent Gelpart on the PECO2, systemic arterial pressure (SAP), maximal velocity of the pulmonary artery (Max Vp), and heart rate (HR) of anesthetized rabbits. A2: open lung. Open lung preparations were used for the experiments investigating the effects of intramediastinal balloon catheter inflation-induced changes in the blood flow volume of the pulmonary artery with or without altered artificial respiration parameters, obstruction of the right bronchus, or the administration of 100% FIO2 on the PECO2, SAP, Max Vp, and HR of anesthetized rabbits. The Max Vp and HR data were obtained using an echocardiographic system. An echocardiographic probe was fixed in place on the left margin of the sternum in the 3rd or 4th intercostal space. A representative color Doppler image is shown in C. The red image shows aortic blood flow, and the blue image represents pulmonary artery blood flow. SAP was recorded using an arterial catheter inserted in the femoral artery, a pressure transducer, and a computer. Physiological saline solution (PSS) was administered at a rate of 18 ml/h during the experiments. The outer diameters of the rabbits’ pulmonary arteries did not change significantly during the experiments.

Fig. 2. A–C: experimental procedure and representative recordings of the changes in respiratory carbon dioxide pressure and color Doppler echocardiography. Pulmonary blood flow on the end-expiratory CO2 gas pressure (PICO2) was continuously recorded using a CO2 gas monitor and a CO2 sensor kit connected to a tracheostomic cannula (A). A representative PICO2 recording is shown in B. A1: closed lung. Closed lung preparations were used for the experiments evaluating the effects of: 1) changes in the hematocrit (Ht) value, 2) the administration of a single iv injection of the cell surface F1/FO ATP synthase inhibitor piceatannol or the CA IV inhibitor acetazolamide (ACZ), 3) the administration of a single iv injection of isoprenaline (ISP) in the presence or absence of phentolamine and/or propranolol, or 4) lung emboli produced by the administration of a single iv injection of the collagen-based embolic agent Gelpart on the PECO2, systemic arterial pressure (SAP), maximal velocity of the pulmonary artery (Max Vp), and heart rate (HR) of anesthetized rabbits. A2: open lung. Open lung preparations were used for the experiments investigating the effects of intramediastinal balloon catheter inflation-induced changes in the blood flow volume of the pulmonary artery with or without altered artificial respiration parameters, obstruction of the right bronchus, or the administration of 100% FIO2 on the PECO2, SAP, Max Vp, and HR of anesthetized rabbits. The Max Vp and HR data were obtained using an echocardiographic system. An echocardiographic probe was fixed in place on the left margin of the sternum in the 3rd or 4th intercostal space. A representative color Doppler image is shown in C. The red image shows aortic blood flow, and the blue image represents pulmonary artery blood flow. SAP was recorded using an arterial catheter inserted in the femoral artery, a pressure transducer, and a computer. Physiological saline solution (PSS) was administered at a rate of 18 ml/h during the experiments. The outer diameters of the rabbits’ pulmonary arteries did not change significantly during the experiments.
α-blocker, phenolamine mesilate (31 μg/kg iv; Novartis Pharma, Tokyo, Japan), and/or a β-blocker, propranolol hydrochloride (94 μg/kg iv; AstraZeneca, Osaka, Japan). Next, to examine the roles played by cell surface, but not mitochondrial, F1/F0 ATP synthase and/or cell surface CA IV in pulmonary blood flow-mediated CO2 gas excretion in the lungs, we studied the dose-dependent effects of a cell surface F1/F0 ATP synthase inhibitor, piceatannol (0.5–1.6 mg/kg iv) (25); a CA IV inhibitor, acetazolamide (ACZ) (1.0–3.1 mg/kg iv) (13); and a selective cell surface F1/F0 ATP synthase antibody (33 μg/kg, administered from the tip of a pulmonary artery catheter; Millipore, Billerica, MA) on the PeCO2, Max Vp, SAP, and HR of anesthetized rabbits.

To investigate the effects of reducing the rabbits’ Ht levels (as a clinical model of anemia) on their PeCO2, Max Vp, SAP, and HR, we extracted 10 ml of arterial blood from the femoral artery, before infusing 10 ml of PSS in the femoral vein. This procedure was performed 10 times over several minutes. The extracted systemic arterial blood samples were subjected to blood gas analysis. After 100 ml of arterial blood had been extracted from the anesthetized rabbits, we evaluated the effects of administering 10 ml of HCO3− solution (0.25 mM HCO3−; Ajinomoto Pharma, Tokyo, Japan) in the femoral vein on their PeCO2, Max Vp, SAP, and HR. In addition, to evaluate the effects of blood viscosity on the anemia-induced changes in the rabbits’ PeCO2, Max Vp, SAP, and HR, we measured the viscosity of heparinized rabbit blood with several Ht levels at 25°C using a Vibro viscometer (SV-1A; A&D Instruments, Tokyo, Japan). To assess the roles of red blood cells in the exchange of CO2 gas in the lungs, the effects of the administration of the same volume of heparinized arterial blood or PSS (3 ml) from the tip of a pulmonary artery catheter on the changes in the rabbits’ PeCO2, Max Vp, SAP, and HR were also examined.

To develop a clinical model of lung embolism, we administered 1 or 3 ml of a collagen-based embolic agent (Gelpart; Nihonkayaku, Tokyo, Japan) in the femoral veins of anesthetized rabbits and then evaluated its effects on the rabbits’ PeCO2, Max Vp, SAP, and HR. Finally, to examine the effects of ventilation-perfusion mismatch (1) on the pulmonary blood flow-dependent modification of the PeCO2 in anesthetized rabbits, we injected 1 ml of the collagen-based embolic agent (1 mm diameter) in the right bronchus and then investigated the effects of maximal intramediastinal balloon catheter inflation on the rabbits’ PeCO2, Max Vp, SAP, and HR.

After the lung embolism and bronchial obstruction experiments, the rabbits’ lung tissues were collected and fixed in 10% formalin solution for 24 h before being used to histologically evaluate the number of lung emboli or the extent of bronchial obstruction. The specimens were then dehydrated through a graded series of ethanol and embedded in paraffin in a routine manner, before 3- to 4-μm-thick tissue sections were processed using hematoxylin-eosin stain. The sections were examined using a light microscope (BZ-9000; Keyence, Tokyo, Japan) and photographed.

**Drugs**

All salts were obtained from Wako (Tokyo, Japan). Angiostatin, piceatannol, and ACZ were purchased from Sigma (St. Louis, MO). The piceatannol was diluted with ethanol. The concentration of ethanol used did not affect the biological viability of the cultured EC. Drug concentrations are expressed as the final concentration in the culture plate.

**Statistical Analysis**

All results are expressed as means ± SE. Statistical significance was analyzed using the Student’s t-test for unpaired observations or one-way ANOVA followed by Duncan’s post hoc test, as appropriate. P < 0.05 was considered statistically significant.

**RESULTS**

**Effects of Maximal Intramediastinal Balloon Catheter Inflation-Induced Reductions in Pulmonary Blood Flow on the PeCO2, Max Vp, SAP, and HR of Anesthetized Rabbits**

We first investigated the effects of maximal intramediastinal balloon catheter inflation-induced reductions in pulmonary blood flow on the PeCO2 of anesthetized rabbits. The gradual reduction in pulmonary blood flow caused by the inflation of the intramediastinal balloon catheter (Fig. 3A) led to a significant reduction in the Max Vp and a concomitant fall in the PeCO2 (Fig. 3A). The reduction in the PeCO2 occurred quickly after the inflation of the balloon (~10 s) (Fig. 3A, right). The maximal inflation of the balloon catheter caused the Max Vp to fall to around 0.48 m/s (control ~0.90 m/s) and the PeCO2 to decrease to ~9 mmHg (control ~34 mmHg). During these experiments, no significant difference in arterial PaO2, Paco2, or pH was observed between the control conditions (before the experiment) and the maximal intramediastinal inflation of the balloon (Fig. 3A). In addition, the mixed venous Paco2 values of the pulmonary artery blood samples collected before and after the inflation of the balloon did not differ significantly [before, 45.7 ± 1.3 mmHg (n = 5) vs. after, 45.1 ± 0.6 mmHg (n = 4), not significant]. Thus, the mixed partial pressure of the CO2 entering the pulmonary circulation did not alter significantly after the inflation of the balloon. The above findings are summarized in Fig. 3B (n = 4).

The cross-sectional area of the point of the pulmonary artery at which Max Vp was measured (Fig. 3C) did not change significantly during the experiments, so according to the abovementioned continuity equation the reduction in Max Vp was probably the result of a reduction in blood flow in the pulmonary artery (7). In addition, the continuity equation also suggests that the flow velocity gradient in the pulmonary arterioles was greater than that in the pulmonary artery, which would have resulted in increased shear stress within the pulmonary arterioles.

An almost linear relationship was detected between the normalized Max Vp and PeCO2 values of the anesthetized rabbits (Fig. 3B), which were maintained under a constant respiratory frequency and tidal volume to minimize the extent of any ventilation-perfusion mismatch. To further evaluate the influence of ventilation-perfusion mismatch (1) on the relationship between Max Vp and PeCO2, we investigated the effects of changes in respiratory frequency and tidal volume on the PeCO2 and Max Vp of the anesthetized rabbits. Representative findings are shown in Fig. 3C. Reducing the rabbits’ tidal volume from 40 ml/breath to 20 ml/breath, as was done in several recent rabbit studies (10, 15, 20), significantly increased their PeCO2 values. However, the reduction in the PeCO2 induced by maximal intramediastinal balloon catheter inflation did not differ significantly between tidal volumes of 40 and 20 ml/breath. In the rabbits in which the tidal volume was reduced to 20 ml/breath, the reductions in the PeCO2 (−61%) and Max Vp (−62%) observed after maximal intramediastinal balloon catheter inflation were quite similar to those seen in the controls (Fig. 3C). In addition, no significant differences in blood gas levels were detected between tidal volumes of 40 and 20 ml/breath.
Examination of the Roles of Pulmonary Blood Flow Through Small Pulmonary Arteries or Arterioles in the Regulation of PECO2 and Max Vp in Anesthetized Rabbits

To examine the roles of pulmonary blood flow through small pulmonary arteries or arterioles in the regulation of PECO2 and Max Vp, we first inserted pulmonary artery catheters in the small pulmonary arteries of anesthetized rabbits and then evaluated the changes in their PECO2 and Max Vp values. Figure 4A shows representative recordings of the rabbits' PECO2 and Max Vp values, as well as a representative echocardiogram. In each case, a catheter was inserted in a small pulmonary artery and was then occluded at the right branch of the pulmonary artery (Fig. 4A, right). The PECO2 fell rapidly in accordance with the degree of stenosis induced in the target arteries. However, the blood flow velocity of the main pulmonary artery did not change significantly.

To examine the roles of cell surface F1/FO ATP synthase in the pulmonary arterioles in the exchange of CO2 gas in the lungs, we next evaluated the effects of the administration of a selective cell surface F1/FO ATP synthase antibody (Millipore) from the tip of a pulmonary artery catheter on the PECO2 and Max Vp, and on the maximal intramediastinal balloon catheter inflation-mediated reduction in the PECO2 in anesthetized rabbits. Figure 4B shows representative recordings of the rabbits' PECO2 and Max Vp values. The administration of the cell surface F1/FO ATP synthase antibody caused a gradual reduc-

Fig. 3. A: representative recordings of the effects of the changes in pulmonary blood flow volume produced by the intramediastinal inflation of a balloon catheter on the PECO2 and Max Vp of anesthetized rabbits. The Max Vp data are shown as the absolute values calculated by the echocardiographic system. Right, expanded version of the section of the tracing surrounded by a square on the left. The arrow indicates the point at which the 3rd stage of balloon inflation was started. The lowest box shows the Pao2, PacO2, and pH data obtained during the control period (before the inflation of the balloon) and after the maximal intramediastinal inflation of the balloon (n = 4). NS, not significant. B: summary of our findings regarding the effects of changes in pulmonary blood flow volume on the %PECO2 (the ordinate) and %Max Vp (the abscissa) of anesthetized rabbits (n = 4). The ordinate and abscissa show the relative changes in the rabbits' PECO2 and Max Vp normalized to the maximal PECO2 and Max Vp values observed before each experiment, respectively. The red line indicates the relationships between %Max Vp (the ordinate) and the mixed venous PCO2 (the ordinate) seen before and after the maximal intramediastinal inflation of the balloon. *P < 0.05 and **P < 0.01, significant difference between each value. C: representative recordings of the maximal intramediastinal balloon catheter inflation-mediated changes in the PECO2 and Max Vp of anesthetized rabbits with artificial respiratory parameters of 40 ml/breath and 20 breaths/min (control) or 20 ml/breath and 30 breaths/min (lower tidal volume). The lowest box shows the Pao2, PacO2, and pH data obtained in the experiments involving artificial respiratory parameters of 40 ml/breath and 20 breaths/min (control) or 20 ml/breath and 30 breaths/min (n = 4).
tion in the \( P_{\text{ECO}_2} \) (from 32 to 28 mmHg) but did not induce a significant change in the Max Vp (from 0.60 to 0.58 m/s). In addition, the maximal intramediastinal balloon catheter inflation-mediated reduction in the \( P_{\text{ECO}_2} \) was significantly ameliorated by treatment with the F1/FO ATP synthase antibody (\( P_{\text{ECO}_2} \): from 32 to 15 mmHg vs. from 30 to 20 mmHg). However, no significant change in the Max Vp was observed after the administration of the antibody (0.60 vs. 0.58 m/s).

Finally, after inserting pulmonary artery catheters in anesthetized rabbits, we evaluated the roles of red blood cells in gas exchange in the lungs. Figure 4C shows representative recordings of the effects of the administration of heparinized arterial blood (3 ml) or heparinized PSS (3 ml) on the \( P_{\text{ECO}_2} \) and Max Vp of anesthetized rabbits subjected to pulmonary artery catheterization-mediated stenosis of the small pulmonary arteries. The arrows show the points at which heparinized blood and saline solution was administered.

\[ \text{Max Vp: 0.66} \quad 0.60 \quad 0.61 \quad 0.60 \quad (\text{m/s}) \]

\[ \text{Heparinized arterial blood 3mL} \]

\[ \text{Heparinized Saline solution 3mL} \]

\[ \text{Max Vp: 0.60} \quad 0.40 \quad 0.39 \quad (\text{m/s}) \]

Fig. 4. A: representative recordings of the effects of pulmonary artery catheterization-mediated stenosis of the smaller pulmonary arteries or arterioles on the \( P_{\text{ECO}_2} \) and Max Vp of anesthetized rabbits. The arrow indicates the point to which the catheter was inserted. The white arrow shows the stenosis produced by the pulmonary artery catheter. B: representative recordings of the effects of the administration of a selective cell surface F1/FO ATP synthase antibody (33 \( \mu \)g/kg) on the \( P_{\text{ECO}_2} \) and Max Vp of anesthetized rabbits. The green square indicates the maximal intramediastinal inflation of the balloon catheter. The arrow shows the point at which the antibody was administered. C: representative recordings of the effects of the administration of heparinized arterial blood (3 ml) or heparinized PSS (3 ml) on the \( P_{\text{ECO}_2} \) and Max Vp of anesthetized rabbits subjected to pulmonary artery catheterization-mediated stenosis of the small pulmonary arteries. The arrows show the points at which heparinized blood and saline solution was administered.

Dose-Dependent Effects of Piceatannol or Acetazolamide on the \( P_{\text{ECO}_2} \), Max Vp, SAP, and HR of Anesthetized Rabbits

To confirm the involvement of cell surface F1/FO ATP synthase and CA type IV in pulmonary blood flow-mediated CO2 gas excretion in the rabbit lung, we investigated the effects of a cell surface F1/FO ATP synthase inhibitor, piceatannol (25), and a CA IV inhibitor, ACZ (16), on the \( P_{\text{ECO}_2} \) and SAP of anesthetized rabbits. The administration of a single intravenous injection of piceatannol (0.5 or 1.6 mg/kg) or ACZ (1.0 or 3.1 mg/kg) produced dose-dependent reductions in the \( P_{\text{ECO}_2} \), but did not affect SAP or the Max Vp (Fig. 5, A and B). These findings are summarized in Fig. 5, C and D (\( n = 4 \)). The intravenous administration of piceatannol or ACZ caused dose-dependent reductions in the \%\( P_{\text{ECO}_2} \) while having little or no
Dose-dependent responses for Acetazolamide

Dose-dependent responses for Acetazolamide

Fig. 5. A: representative recordings of the effects of the administration of a single iv injection of a cell surface F1/FO ATP synthase inhibitor, piceatannol [0.5 (left) or 1.6 (right) mg/kg] on the PECO2, Max Vp, and SAP of anesthetized rabbits. B: representative recording of the effects of the administration of a single iv injection of a CA IV inhibitor, ACZ (1.0 or 3.1 mg/kg), on the PECO2, Max Vp, and SAP of anesthetized rabbits. C: summary of our findings regarding the dose-dependent effects of piceatannol [0.5 (left) or 1.6 (right) mg/kg iv] on the %PECO2, %SAP, %HR, and %Max Vp of anesthetized rabbits (n = 4). The ordinates show the time courses of the abovementioned parameters before and after the administration of the drug. *P < 0.05 and **P < 0.01, significant difference from each value.

Effects of Drug-Induced Increases in Pulmonary Blood Flow on the PECO2, Max Vp, SAP, and HR of Anesthetized Rabbits

To examine whether a drug-induced increase in pulmonary blood flow can lead to a Max Vp-dependent rise in the PECO2, we investigated the effects of the administration of a single intravenous injection of a β-agonist, ISP (0.31 μg/kg) (24), on the PECO2, Max Vp, SAP, and HR of anesthetized rabbits in the presence or absence of an α-blocker, phentolamine (31 μg/kg) (18), and a β-blocker, propranolol (94 μg/kg) (9). Representative recordings are shown in Fig. 6, A and B. The intravenous injection of ISP (0.31 μg/kg) produced a marked reduction in SAP and a simultaneous increase in HR (Fig. 6A). Marked increases in the Max Vp and PECO2 were also observed, which might have been caused by the ISP-induced increase in the rabbits’ HR. These findings are summarized in Fig. 6C. Pretreatment with phentolamine (31 μg/kg) and propranolol (94 μg/kg) for more than 5 min significantly ameliorated the ISP-induced reduction in the SAP (Fig. 6B). On the other hand, ISP-induced increases in the Max Vp and PECO2 were still observed in the rabbits that were pretreated with phentolamine and propranolol. As shown in Fig. 6, C and D, pretreatment with the abovementioned substances significantly suppressed (P < 0.05, n = 4), but did not completely abolish, the ISP-induced increases in %HR and %Max Vp (Fig. 6D). The ISP-induced increase in the PECO2 might have been related to the positive ino- and chronotropic effects of the drug on the rabbits’ hearts, which could have led to significant increases in the blood flow of the pulmonary arteries.
Next, to confirm that cell surface F1/FO ATP synthase-dependent CO2 gas excretion in the pulmonary arterioles was involved in the ISP-induced increase in the PECO2, we examined the effects of a selective cell surface F1/FO ATP synthase antibody and ACZ on the ISP-induced increase in the PECO2. Figure 6, E and F, shows representative recordings of the effects of the antibody and ACZ, respectively, on the ISP-induced increase in the PECO2. The administration of the cell surface F1/FO ATP synthase antibody from the tip of a pulmonary artery catheter caused a gradual decrease in the PECO2 of the anesthetized rabbits. In the antibody-treated rabbits, the administration of a single intravenous injection of ISP (0.31 µg/kg) produced a smaller increase in the PECO2 than it produced in the control rabbits (not treated with the antibody) even though ISP caused a similar increase in the Max Vp before (control) and after the administration of the antibody. Similar experiments in which ACZ (3.1 µg/kg) was administered from the tip of a pulmonary artery catheter were also conducted. Figure 6F shows representative recordings of the PECO2 and Max Vp. The administration of ACZ caused a marked decrease in the PECO2 of the anesthetized rabbits. In the ACZ-treated rabbits, the administration of a single intravenous injection of ISP (0.31 µg/kg) induced a smaller (but significant) increase in the PECO2 than was seen in the controls, even though similar increases in the Max Vp of the anesthetized rabbits were produced by ISP before and after the ACZ treatment (from 0.68 to 1.00 vs. 0.66 to 0.96 m/s).

Effects of Reductions in the Ht Level on the PECO2, Max Vp, SAP, HR, and Blood Viscosity of Anesthetized Rabbits

Next, we investigated whether changes in the Ht level were associated with significant changes in the PECO2 in anesthetized rabbits. When the Ht level was reduced to around 28% (control...
In contrast, when the Ht concentrations of the same rabbits were reduced from 24 to 20% using the same procedure, as shown in Fig. 7B, neither the $P_{E_{CO_2}}$ nor SAP changed significantly (Fig. 7D). In agreement with these findings, the rabbits’ blood viscosity was not significantly altered by reducing their Ht level from 24 to 20% (Fig. 7D). No significant differences in blood gas levels were detected between Ht levels of 70 and 52% (Fig. 7D). These findings are summarized in Fig. 7D ($n = 4$). In addition, reducing the %Ht from 70 to 52% did not have a significant effect on the $P_{E_{CO_2}}$.

When the Ht levels of the same rabbits were reduced to $\sim$16% (47%) via the extraction of 100 ml arterial blood and the infusion of the same volume of PSS (18.8 ± 1.0 mM HCO₃⁻), the
intravenous infusion of 10 ml HCO₃ solution (12.5 mM HCO₃⁻ in a total volume of 500 ml; Ajinomoto Pharma) produced a rapid and significant increase in the PSCO₂, without causing significant changes in SAP or the Max Vp (Fig. 7E). These findings are summarized in Fig. 7F (n = 4). In the rabbits with the lowest %Ht levels (~47%), the intravenous administration of a small amount of HCO₃ caused a significant increase in the PSCO₂.

Effects of Pulmonary Emboli on the PSCO₂, Max Vp, SAP, and HR of Anesthetized Rabbits

To examine whether the continuous measurement of PSCO₂ in patients maintained under a constant respiratory frequency and tidal volume can be used to evaluate relative changes in pulmonary blood flow volume without echocardiography, we next investigated the effects of Gelpart (19) (a collagen-based embolic agent, 1 mm diameter)-induced emboli in the pulmonary artery on the PSCO₂, Max Vp, SAP, and HR of anesthetized rabbits. The intravenous injection of 1 ml of Gelpart caused a gradual and significant reduction in the PSCO₂ without inducing significant changes in HR or SAP, which is compatible with the observed fall in the Max Vp (Fig. 8A). These findings are summarized in Fig. 8B (n = 4). Gelpart-induced emboli were clearly observed in the small pulmonary arteries (functional resistance vessels, 50–150 μm outer diameter) (Fig. 8, E1 and E2).

When the amount of Gelpart administered was increased from 1 to 3 ml, the rabbits’ SAP decreased rapidly, and their HR fell significantly, which might have been related to a Gelpart-induced downregulation of cardiac function (Fig. 8C). In addition, their %PSCO₂ and %Max Vp exhibited marked simultaneous reductions (Fig. 8D), and a corresponding increase in the number of Gelpart-induced emboli in the small

![Graphs and images showing the effects of emboli on PSCO₂, Max Vp, SAP, and HR.](http://ajplung.physiology.org/)
pulmonary arteries was also seen (Fig. 8, E3 and E4) [no. of emboli/mm²: 1 ml Gelpart: 2.5 ± 0.4 (n = 6) vs. 3 ml Gelpart: 7.0 ± 0.5 (n = 6), P < 0.01]. These findings are summarized in Fig. 8F (n = 6).

Effects of Bronchial Obstruction or the Administration of 100% FiO₂ on the PₚCO₂ and Max Vp of Anesthetized Rabbits

Next, to evaluate whether ventilation-perfusion mismatch or hypoxic vasoconstriction was directly responsible for the maximal intramediastinal balloon catheter inflation-mediated reductions in the PₚCO₂ observed in the anesthetized rabbits, we investigated the effects of the intrabronchial administration of collagen gel (Gelpart, 1 mm diameter) or 100% FiO₂ on the PₚCO₂ and Max Vp. In the rabbits in which 1 ml of Gelpart was administered in the right bronchus, which was expected to produce a ventilation-perfusion mismatch in the affected lung, the maximal intramediastinal inflation of the balloon caused similarly significant reductions in the Max Vp and PₚCO₂ to those seen in the controls (Fig. 3B). A representative tracing is shown in Fig. 9A, a summary of the results is shown in Fig. 9B, and representative histological findings are shown in Fig. 9C. The large bronchus was completely obstructed by the Gelpart (Fig. 9C).

Next, we investigated the effects of 100% FiO₂ on the maximal intramediastinal balloon catheter inflation-mediated reduction in the PₚCO₂. Representative tracings are shown in

![Diagram A](image1)

![Diagram B](image2)

![Diagram C](image3)

![Diagram D](image4)

**Blood gas determination**

- **PaO₂**: 153.5 ± 4.7 mmHg vs. 266.2 ± 5.6 mmHg (100% FiO₂ vs. control)
- **PaCO₂**: 40.0 ± 1.0 mmHg vs. 36.8 ± 0.5 mmHg (100% FiO₂ vs. control)
- **pH**: 7.43 ± 0.02 vs. 7.43 ± 0.03 (100% FiO₂ vs. control)

**Table:**

<table>
<thead>
<tr>
<th>Condition</th>
<th>PaO₂ (mmHg)</th>
<th>PaCO₂ (mmHg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>153.5 ± 4.7</td>
<td>40.0 ± 1.0</td>
<td>7.43 ± 0.02</td>
</tr>
<tr>
<td>100% FiO₂</td>
<td>266.2 ± 5.6</td>
<td>36.8 ± 0.5</td>
<td>7.43 ± 0.03</td>
</tr>
</tbody>
</table>

*NS: Not significant, **P < 0.01*

Fig. 9: A: representative tracings of the effects of maximal intramediastinal balloon catheter inflation on the PₚCO₂ and Max Vp of rabbits before and after obstruction of the right bronchus via the administration of 1 ml Gelpart. B: summary of our findings regarding the effects of maximal intramediastinal balloon catheter inflation on the %PₚCO₂ and %Max Vp of anesthetized rabbits (n = 4). The ordinate and abscissa show the same items as in Fig. 3B. The black and blue lines denote the data obtained in the control conditions (before the inflation of the balloon) and after maximal inflation of the balloon catheter, respectively. **P < 0.01, significant difference between each value. C: representative histological findings of the rabbits’ lungs before and after the obstruction of the right bronchus via the administration of 1 ml of Gelpart. The arrowheads indicate the large bronchi. The marker represents 500 μm. D: representative tracings of the effects of 100% FiO₂ on maximal intramediastinal balloon catheter inflation-mediated reductions in the PₚCO₂ and Max Vp of anesthetized rabbits. The lower box demonstrates the PaO₂, PaCO₂, and pH data obtained in the control and 100% FiO₂ experiments (n = 4). **P < 0.01, significant difference between each value.
significant and rapid effects on the \( P_{\text{ECO}_2} \) in anesthetized rabbits. Thus, we have confirmed that pulmonary blood flow has a gradient that is greater in pulmonary arterioles than in the pulmonary vasculature. However, as mentioned in the Introduction, critical reductions in pulmonary blood flow caused by decreases in the \( P_{\text{ECO}_2} \) were not significantly affected. Treatment with the antibody also significantly ameliorated the effects of maximal intramediastinal balloon catheter inflation on the \( P_{\text{ECO}_2} \). In addition, gradual stenosis of small pulmonary arteries or arterioles via the insertion of a pulmonary artery catheter induced a rapid and concomitant reduction in the \( P_{\text{ECO}_2} \), even though the Max Vp was not significantly affected. These findings strongly support the hypothesis that pulmonary arteriole cell surface \( F_1/F_0 \) ATP synthase is involved in the observed changes in the \( P_{\text{ECO}_2} \).

In conclusion, the findings of the present in vivo experiments strongly support the proposed new concept (13) of \( \text{CO}_2 \) gas exchange in pulmonary arteriolar EC, i.e., pulmonary blood flow-mediated cell surface \( F_1/F_0 \) ATP synthase and CA IV-dependent \( \text{CO}_2 \) gas excretion from arteriolar EC. This conclusion is compatible with the clinical findings that patients with serious anemia exhibit few or no symptoms of reduced \( \text{CO}_2 \) transport-mediated acidosis (8). In addition, the proposed concept (13) can explain why the physiological conversion of \( \text{HCO}_3^- \) to \( \text{CO}_2 \) was observed in isolated rat lungs that were perfused with a red blood cell-free Krebs-Ringer bicarbonate solution (4). In fact, in the present study the administration of red blood cell-free PSS to anesthetized rabbits via the tip of an occluded pulmonary artery catheter produced a similar increase in the \( P_{\text{ECO}_2} \) to that seen when the same amount of heparinized pulmonary artery blood was administered in the same manner to the same rabbits (Fig. 4C). The validity of the proposed new concept is also supported by the findings of previous studies in which critical reductions in pulmonary blood flow caused by decreased cardiac output resulted in lower end-tidal carbon dioxide concentrations in animal or clinical models (1, 2, 22). These findings strongly suggest that shear stress stimulation-mediated \( \text{CO}_2 \) gas excretion from pulmonary arteriolar EC mainly occurs in physiological conditions, and the detection of red blood cell-mediated \( \text{CO}_2 \) gas excretion might also support the observed effects.

**DISCUSSION**

**In Vivo Experimental Support for the New Concept of Pulmonary Arterial Blood Flow-Mediated \( \text{CO}_2 \) Gas Excretion in the Lungs**

In the present study, to address the validity of our proposed concept of \( \text{CO}_2 \) gas excretion from pulmonary arteriolar EC in the lungs, we first investigated the effects of maximal intramediastinal balloon catheter inflation-induced reductions in pulmonary blood flow on the \( P_{\text{ECO}_2} \), using anesthetized rabbits that were maintained under a constant respiratory frequency and tidal volume. As a result, we detected a linear relationship between the normalized Max Vp and \( P_{\text{ECO}_2} \). A clear relationship was observed between these parameters at %Max Vp values ranging from 100% (control) to ~50%. Lowering the rabbits' Max Vp produced a rapid (~10 s) and concomitant reduction in their \( P_{\text{ECO}_2} \). In addition, the mixed venous \( P_{\text{CO}_2} \) values seen before and after the inflation of the balloon catheter did not differ significantly. It is possible that the lower \( P_{\text{ECO}_2} \) values seen after the drop in pulmonary artery blood flow were directly caused by ventilation-perfusion mismatch or hypoxic pulmonary vasoconstriction. However, these possibilities were ruled out by the fact that the changes in the \( P_{\text{ECO}_2} \) were rapid (~10s) and reproducible, and the drop in pulmonary arterial blood flow did not have significant effects on venous \( P_{\text{CO}_2} \) (Fig. 3B). In addition, the rabbits subjected to bronchial obstruction, which is considered to produce ventilation-perfusion mismatch in the lungs, maximal intramediastinal balloon catheter inflation produced similar reductions in the \( P_{\text{ECO}_2} \) to those seen in the controls (without bronchial obstruction) (Fig. 9A). Thus, we have confirmed that pulmonary blood flow has significant and rapid effects on the \( P_{\text{ECO}_2} \) in anesthetized rabbits. Studies involving animal or clinical models have found that marked reductions in cardiac output (and the subsequent drops in pulmonary blood flow) led to lower end-tidal carbon dioxide concentrations (1, 2, 22). However, the detailed mechanisms responsible for these processes remain unknown.

In the present study, we did not obtain any in vivo experimental data that enabled us to determine whether the observed effects arose from a particular arteriolar portion of the pulmonary vasculature. However, as mentioned in the Introduction, according to one of the basic equations of fluid mechanics, the continuity equation for Newtonian fluids, the flow velocity gradient is greater in pulmonary arterioles than in the pulmonary artery providing the pulmonary blood flow volume remains constant. Thus, changes in the flow velocity of the pulmonary arterioles might have more significant effects than variations in the flow velocity of the pulmonary artery. These findings might contribute to clarifying the locations at which the observed effects occurred.

On the other hand, in the present study treatment with a selective cell surface \( F_1/F_0 \) ATP synthase antibody produced a gradual reduction in the \( P_{\text{ECO}_2} \) but did not affect the Max Vp. The administration of 100% \( \text{FIO}_2 \) caused a significant reduction in the \( P_{\text{ECO}_2} \) (from 37 to 26 mmHg). In addition, gradual stenosis of small pulmonary arteries or arterioles via the insertion of a pulmonary artery catheter induced a rapid and concomitant reduction in the \( P_{\text{ECO}_2} \), even though the Max Vp was not significantly affected. Pretreatment with the rabbits' pulmonary arterioles with the cell surface \( F_1/F_0 \) ATP synthase antibody or the cell surface CA inhibitor (16) AZS significantly reduced the ISP-induced increases in the \( P_{\text{ECO}_2} \). We also confirmed that the intravenous administration of AZS or the cell surface (but not mitochondrial) \( F_1/F_0 \) ATP synthase inhibitor (25) piceatannol caused significant dose-dependent reductions in the \( P_{\text{ECO}_2} \) while having little or no effect on SAP, HR, or the Max Vp. Cell surface \( F_1/F_0 \) ATP synthase and CA IV are known to localize in the EC of pulmonary arterioles (11). Thus, the pharmacological findings we obtained with ISP, piceatannol, and AZS might help to clarify the locations at which the observed effects occurred.

In the present study, we demonstrated that reducing the %Ht from ~70 to 47% had no significant effect on the %PECO2 of anesthetized rabbits. In contrast, lowering the %Ht from 100% to ~70% produced significant reductions in SAP and the \( P_{\text{ECO}_2} \), together with a concomitant fall in blood viscosity (Fig. 7C). The decrease in blood viscosity would have led to marked reductions in systemic vascular resistance and the shear stress placed on the EC in the pulmonary arterioles. The reduction in shear stress might in turn have contributed to the significant fall in the \( P_{\text{ECO}_2} \) seen in the anesthetized rabbits.

On the other hand, in the present study treatment with a selective cell surface \( F_1/F_0 \) ATP synthase antibody produced a linear relationship between the normalized Max Vp and \( P_{\text{ECO}_2} \). A clear relationship was observed between these parameters at %Max Vp values ranging from 100% (control) to ~50%. Lowering the rabbits' Max Vp produced a rapid (~10 s) and concomitant reduction in their \( P_{\text{ECO}_2} \). In addition, the mixed venous \( P_{\text{CO}_2} \) values seen before and after the inflation of the balloon catheter did not differ significantly. It is possible that the lower \( P_{\text{ECO}_2} \) values seen after the drop in pulmonary artery blood flow were directly caused by ventilation-perfusion mismatch or hypoxic pulmonary vasoconstriction. However, these possibilities were ruled out by the fact that the changes in the \( P_{\text{ECO}_2} \) were rapid (~10s) and reproducible, and the drop in pulmonary arterial blood flow did not have significant effects on venous \( P_{\text{CO}_2} \) (Fig. 3B). In addition, the rabbits subjected to bronchial obstruction, which is considered to produce ventilation-perfusion mismatch in the lungs, maximal intramediastinal balloon catheter inflation produced similar reductions in the \( P_{\text{ECO}_2} \) to those seen in the controls (without bronchial obstruction) (Fig. 9A). Thus, we have confirmed that pulmonary blood flow has significant and rapid effects on the \( P_{\text{ECO}_2} \) in anesthetized rabbits. Studies involving animal or clinical models have found that marked reductions in cardiac output (and the subsequent drops in pulmonary blood flow) led to lower end-tidal carbon dioxide concentrations (1, 2, 22). However, the detailed mechanisms responsible for these processes remain unknown.

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the proposed concept of pulmonary arteriolar EC-mediated CO₂ gas excretion.

Therefore, taking all of the findings obtained in the present study and our previous molecular biological study involving cultured human pulmonary arteriolar EC (13) into consideration, we reaffirm the validity of our proposed concept of CO₂ gas exchange in pulmonary arterioles, which involves a very short period of shear stress stimulation-induced cell surface F₁/FO ATP synthase-dependent H⁺ secretion and the subsequent reaction of H⁺ with HCO₃⁻ in the plasma, resulting in the excretion of CO₂ gas from arteriolar EC.

The proposed concept might be involved in efficient and rapid oxygen absorption by red blood cells from the blood capillaries in the pulmonary circulation (~0.75 s) (14). In addition, based on current evidence, the proposed concept suggests that CO₂ gas exchange through the pulmonary arterioles might ensure that red blood cells have sufficient time to absorb oxygen from pulmonary capillaries.

Clinical Implications of the New Concept

To examine the clinical implications of the new concept, we investigated the effects of reduced Ht levels (a model of anemia) or embolic agent-induced emboli in the pulmonary arterioles of rabbits. Marked anemia, e.g., %Ht values ranging from ~70 to 47%, had no significant effect on the PECO₂, which is compatible with clinical evidence obtained in patients with anemia (8).

In addition, we found that pulmonary emboli caused a gradual and significant reduction in the PECO₂, the extent of which depended on the number of emboli present in the lungs, which is compatible with the reduced Max Vp exhibited by the anesthetized rabbits. Our findings suggest that the continuous measurement of the PECO₂ in patients maintained under a constant respiratory frequency and tidal volume can be used to quickly evaluate changes in pulmonary blood flow. Further investigations will be needed to confirm whether our suggestions regarding the clinical implications of our findings are correct.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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