Response of Intra-acinar Pulmonary Microvessels to Hypoxia, Hypercapnic Acidosis, and Isocapnic Acidosis

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Abstract—To elucidate the differential reactivity of pulmonary microvessels in the acini to hypoxia, excessive CO2, and increased H+, we investigated changes in the diameter of precapillary arterioles, postcapillary venules, and capillaries in isolated rat lungs on exposure to normocapnic hypoxia (2% O2), normoxic hypercapnia (15% CO2), and isocapnic acidosis (0.01 mol/L HCl). Microvascular diameters were precisely examined using a real-time confocal laser scanning luminescence microscope coupled to a high-sensitivity camera with an image intensifier. Measurements were made under conditions with and without indomethacin or Nω-nitro- L-arginine methyl ester to assess the importance of vasoactive substances produced by cyclooxygenase (COX) or NO synthase (NOS) as it relates to the reactivity of pulmonary microvessels to physiological stimuli. We found that acute hypoxia contracted precapillary arterioles that had diameters of 20 to 30 μm but did not constrict postcapillary venules of similar size. COX- and NOS-related vasoactive substances did not modulate hypoxia-elicited arteriolar constriction. Hypercapnia induced a distinct venular dilatation closely associated with vasodilators produced by COX but not by NOS. Arterioles were appreciably constricted in isocapnic acidosis when NOS, but not COX, was suppressed, whereas venules showed no constrictive response even when both enzymes were inhibited. Capillaries were neither constricted nor dilated under any experimental conditions. These findings suggest that reactivity to hypoxia, CO2, and H+ is not qualitatively similar among intra-acinar microvessels, in which COX- and NOS-associated vasoactive substances function differently. (Circ Res. 1998;82:722-728.)

Key Words: acinus ■ hypoxia ■ acidosis ■ cyclooxygenase ■ nitric oxide synthase

In the normal lung, vascular reactivity of pulmonary vessels responding to O2, CO2, and hydrogen ion (H+) is considered exceedingly important in regulating the distribution of pulmonary blood flow, allowing the lung to maintain a reasonable match between ventilation and blood flow. Alveolar hypoxia and increased H+ concentration induced either by alveolar hypercapnia (hypercapnic acidosis) or by isocapnic acidosis (ie, metabolic acidosis) have been shown to evoke active vasoconstriction in the pulmonary circulation.1-7 However, the CO2 molecule, per se, has been presumed to induce pulmonary vasodilatation, as when Viles and Shepherd1,2 found the pressor response of isolated feline lungs to hypercapnic acidosis to be less than that to isocapnic acidosis at the same pH level. Thus, pulmonary vascular response to hypercapnic acidosis may be the net result of vasoconstriction due to increased H+ concentration and vasodilation due to increased CO2 tension. Direct evidence, however, for CO2 molecule-elicited vasodilatation of pulmonary vessels has not been obtained conclusively. Although several groups of investigators1-7 have attempted to produce definite evidence of active vasoconstriction of pulmonary vessels mediated by alveolar hypoxia or by alveolar hypercapnia, the effects of alveolar hypercapnia on pulmonary vessels are less consistent than those of alveolar hypoxia. Additionally, we would place great emphasis on the fact that inquiry has been largely absent in determining whether intra-acinar microvessels, including precapillary arterioles and post-capillary venules, having diameters of 20 to 30 μm can be actively constricted in response to various physiological stimuli.

Vasoactive PGs and NO have recently emerged as important substances modifying pulmonary vessel response to hypoxic stimulation.6-8 However, their significance in HPV in the acini has not been critically evaluated. Furthermore, analysis of the importance of PGs and/or NO in H+ - and CO2-elicited vascular response is very limited for the pulmonary circulation and totally lacking for the intra-acinar microcirculation. In view of these facts, we also intend to shed light on the possible roles of vasodilating PGs, such as prostacyclin (PGI2) and NO, both of which are produced mainly in vascular endothelial cells, in modifying the contraction and/or dilatation of intra-acinar microvessels in intact rat lungs.
Selected Abbreviations and Acronyms
CCD = charge-coupled device
COX = cyclooxygenase
ECMO = extracorporeal membrane oxygenator
HPAEC = human pulmonary artery endothelial cell
HPV = hypoxic pulmonary vasoconstriction
Ht = hematocrit
6-keto-PGF\(_2\alpha\) = 6-ketoprostaglandin F\(_{1\alpha}\)
L-NAME = \(N^\text{\#}\)-nitro-L-arginine methyl ester
NOS = NO synthase
PG = prostaglandin
Ppa = pulmonary arterial pressure

Materials and Methods
Preparation of Isolated Perfused Rat Lungs
We used isolated perfused lungs that were prepared from male pathogen-free Sprague-Dawley rats (8 weeks old) weighing 250 to 300 g (n=112). The isolated perfused lung preparation has been described in detail elsewhere.\(^6\) Briefly, after heparin was administered into the left ventricle, catheters were inserted into the pulmonary artery and left atrium and were secured with strings. A ligature was placed around the aorta to prevent loss of the perfusate into the systemic circulation. The isolated lung was then fixed on a microscopic stage with animals in the supine position and perfused into the systemic circulation. The isolated lung was then fixed on a microscopic stage with animals in the supine position and perfused into the systemic circulation. The perfusate sample (2 mL) collected in the tube containing 3% bovine serum albumin was used as the perfusate, in which the Ht was adjusted to 0.05 by adding fresh blood obtained from donor rats. The trachea was ligated at the end-inspiratory position, and gas exchange was maintained with an ECMO (Merasilox-S, Senko) inserted between the isolated lung and the roller pump. A gas mixture containing 21% \(O_2\) and 5% \(CO_2\) at 8 mL/s, was used as the gas flowing into the ECMO, allowing adjustment of the perfusate \(P_O_2\) to 19±0.4 kPa (142±3 mm Hg), \(P_CO_2\) to 5.1±0.3 kPa (38±2 mm Hg), and pH to 7.4±0.1. A warmed, humidified gas mixture containing the same composition of gases as used for the ECMO was supplied continuously at 16 mL/s to the lung surface to maintain a temperature of 37±0.5°C and to avoid desiccation of the lung surface. Ppa was continuously monitored with a pressure transducer (TP-400TT, Nihon Kohden) connected to a small cannula whose tip was located in the catheter that was inserted into the main pulmonary artery.

Experimental Protocols
After stable Ppa was attained, the gas flowing into the ECMO and the gas blown onto the lung surface were simultaneously switched from the control gas (21% \(O_2\) and 5% \(CO_2\) in \(N_2\)) to the following mixtures: (1) hypoxic-normocapnic gas composed of 2% \(O_2\) and 5% \(CO_2\) in \(N_2\) for HPV to be elicited (perfusate \(P_O_2\) was reduced to 4.5±0.3 kPa [34±2 mm Hg], but perfusate pH was maintained at 7.4); (2) normoxic-hypercapnic gas with 21% \(O_2\) and 15% \(CO_2\) in \(N_2\) for inducing normoxic-hypercapnic acidosis (\(P_CO_2\) increased to 13±0.5 kPa [94±4 mm Hg], and pH decreased to 7.1±0.04); and (3) 1 mL of 1 mol/L HCl slowly administered into the perfusate over 5 minutes under conditions in which the control gas was provided to the ECMO and onto the lung surface to assess the effect of isocapnic acidosis on pulmonary circulation (the final concentration of HCl in the perfusion medium was 0.01 mol/L). The addition of HCl reduced perfusate pH to 7.1±0.1, which was not statistically different from the pH observed at hypercapnic acidosis. After the introduction of hypoxic gas, hypercapnic gas, or HCl, >10 minutes was required for the Ppa to plateau. Therefore, we continuously monitored Ppa for 15 minutes and measured microvascular diameter thereafter (see below). Changes in Ppa from the baseline value under a given experimental condition were used to measure the extent of change in overall vascular resistance occurring in the pulmonary circulation, including intra-acinar microvessels and extra-acinar vessels of a larger size.

To elucidate the importance of the response of vasoactive PGs and NO in the pulmonary circulation to alveolar hypoxia, hypercapnic acidosis, or isocapnic acidosis, each animal group was subdivided into three categories based on the agents used: (1) medication-free group (N group; measurements were made without administration of any agent); (2) indomethacin group (I group; indomethacin in Sigma Chemical Co) was used to restrain a constitutive form of COX (COX-1, and the perfusate concentration of indomethacin was adjusted to 20 \(\mu\)mol/L); (3) L-NAME group (L group; a constitutive form of NOS [endothelial NOS] was inhibited with L-NAME [Sigma], whose concentration in the perfusate was maintained at 100 \(\mu\)mol/L).

Measurements of Pulmonary Microvascular Diameters
To obtain images suitable for precisely estimating the events occurring in pulmonary microvessels, we used a confocal luminescence microscope recently developed in our laboratory.\(^4\) The reflected light or fluorescent emission from the sample was imaged onto a high-sensitivity CCD camera with an image intensifier (Ektapro Intensified Image VSG, Kodak), which can detect even very low fluorescence signals. By incorporating an excitation wavelength of 488 nm emitted from a low-power air-cooled argon ion laser (532-BSA04; output power, 10 mW; Omnichrome) with appropriate fluorochromes, our confocal system enabled us to obtain apparently instantaneous images at 1000 frames/s. The final magnifying power of our system reached \(\times 968\), with a \(\times 40\) objective, on the video screen. The resulting field of view was 105×105 \(\mu\m\), corresponding roughly to a diameter of a single pulmonary microvessel running beside the terminal bronchiole.\(^2\) We registered confocal images at a rate of 250 frames/s by means of a high-speed video analysis system (Ektapro 1000 Processor, Kodak) connected to the image-intensified CCD camera (Ektapro Intensified Imager VSG, Kodak).

To determine microvessel diameter and architecture, we added 200 \(\mu\)L of 5% FITC-dextran with a molecular weight of 145 000 (Sigma) to the reservoir. Defining the edge of the microvessel of interest as the portion exhibiting a steep change of fluorescence signal, we calculated vessel diameters by processing a confocal video image with a computer-assisted digital image–analyzing system (Imaris 4.08, Imaris Co). To discrimininate between precapillary arterioles and postcapillary venules, we added a small quantity of erythrocytes stained with FITC (Sigma) to the perfusion medium and measured their flow direction with the confocal luminescence microscope.

Measurements of PGI\(_2\) and NO-Related Metabolites in the Perfusate
The perfusate sample (2 mL) collected in the tube containing indomethacin and EDTA was centrifuged and acidified with acetic acid and frozen at −80°C until extraction. At extraction, 1 mL of acidified fluid was extracted into 4-mL ethyl acetate by shaking for 1 hour. The ethyl acetate layer was transferred to a second tube, evaporated to dryness under pure \(N_2\) gas, and stored frozen. At assay, the dried extract was reconstituted in the assay buffer. Immunoreactive 6-keto-PGF\(_{1\alpha}\), a stable metabolite of PG\(_I_2\), was determined by ELISA (EIA kit, Cayman Chemical).

As a measure of NO production in the lung, we examined the total concentration of end products of NO metabolism, \(NO_2^-\) and \(NO_3^-\), in the perfusate.\(^3\) The \(NO_2^-/NO_3^-\) level was spectrophotometrically determined by modifying the method of Green et al.\(^4\) Five milliliters of the perfusate was transferred into a vessel containing 5 mL of 5% ammonium chloride and 25 mL of distilled water. The prepared sample was passed through a column packed with copper-plated cadmium, allowing the complete reduction of \(NO_3^-\) to \(NO_2^-\). Ten milliliters of the effluent was mixed with the reagent containing 1
mL of 1% sulfanilamide and 1 mL of 0.1% N-(1-naphthyl)ethylendiamine in 5% phosphoric acid, and the color of the product yielded by the diazotization reaction was measured with a spectrophotometer (US501, Unisoku) at an absorbance wavelength of 540 nm.

**Measurements of pH\_i During Hypercapnia and Isocapnic Acidosis**

Pulmonary artery endothelial cells harvested from human large pulmonary arteries (HPAECS, Kurabo) were cultured in the cell growth medium (Kurabo) supplemented with 10% fetal calf serum, 100 U/mL penicillin G, and 100 \( \mu \text{g/mL} \) streptomycin at 37°C in a humidified atmosphere of 95% O\(_2\)/5% CO\(_2\). Endothelial cells were grown on a 250-mm\(^2\) plate in a tissue culture well (Corning) for 24 hours. Cells thus grown were subcultured with 0.05% trypsin-EDTA (GIBCO) and washed with Dulbecco’s phosphate-buffered saline (Sigma). Endothelial cell monolayers on the plate were loaded with 1% bovine serum albumin containing 2.5 \( \mu \text{mol/L} \) of a fluorescent pH indicator, BCECF (Molecular Probes), at room temperature for 30 minutes. Fluorescence signals emitted from BCECF excited at 450 and 540 nm were measured with a spectrophotometer (US501, Unisoku) at an absorbance wavelength of 530 nm, and the ratio of fluorescence emission at these two wavelengths was used as a measure of pHi. The fluorescence signals were photographed with a high-sensitivity SIT camera (C2400 to 87, Hamamatsu Photonics) connected to an image-processing system (Argus-50/CA II, Hamamatsu Photonics). The plate on which BCECF-loaded cells were placed was transferred to an airtight chamber located on the stage of an inverted microscope (Dpan 300, Nikon), through which the Krebs-Henseleit buffer equilibrated with the control gas containing 21% O\(_2\) and 5% CO\(_2\) (pH\(_j\) 7.4) was slowly perfused at a rate of 0.03 mL/s (MP-3, Rikakikai) in a single passage. The chamber and perfusate temperatures were maintained at 37°C. Changing the equilibrating gas mixture from the control to the hypercapnic gas with 21% O\(_2\) and 15% CO\(_2\), we monitored changes in pHi, for 15 minutes. pHi during hypercapnic acidosis was maintained at \( p\text{H}_i = 7.05 \pm 0.02 \). Subsequently, altering the perfusate in which pHi was adjusted to hypercapnic conditions \( (7.07 \pm 0.01) \) by the addition of appropriate quantities of 1 mol/L HCl via exposure to the control gas, we measured pHi changes caused by isocapnic acidosis again for 15 minutes.

The H\(^+\)-K\(^+\) antiporter nigericin (20 mg/L, Sigma) and the pH-adjusted buffer containing 130 mmol/L KCl were used to maintain pHi, and pH\(_i\) at the same level.\(^{21}\) To obtain the relationship between absolute pHi and fluorescence intensity, BCECF-loaded cells were exposed to a solution containing the agents described above. Under these conditions, the fluorescence ratios at 450 and 490 nm were measured in the pH range from 6.6 to 7.7, in which fluorescence ratios and pH were linearly related, thus allowing a simple conversion of BCECF fluorescence signals to corresponding pH\(_i\) values.

**Statistical Evaluation**

Statistical differences in the results obtained for different experimental conditions were generally judged in terms of one-way ANOVA followed by multiple comparison Scheffe’s test. Comparison of Ppa before and after introducing hypoxia, hypercapnia, or isocapnic acidosis was made by the paired \( t \) test or the Wilcoxon test. The effect of indomethacin on vasoactive PG genesis and that of L-NAME on NO production before and after a given stimulation were judged by virtue of the paired \( t \) test or the Wilcoxon test, as well. The differences in transitional changes in pH\(_i\), during hypercapnic acidosis and isocapnic acidosis were examined on the basis of the Hotelling \( T^2 \) statistic and the unpaired \( t \) test. Values are presented as mean±SD, and \( P<0.05 \) was taken to be statistically significant.

**Results**

**Basic Hemodynamic Characteristics in the Pulmonary Circulation**

Baseline Ppa before stimulation with hypoxia, hypercapnic acidosis, and isocapnic acidosis in the absence of medications averaged 13, 12, and 11 mm Hg, respectively, with no difference among values (Table 1). The diameters of precapillary arterioles in the acini under conditions with no stimulation ranged from 20 to 30 \( \mu \text{m} \), and did not differ from those of postcapillary venules. Baseline capillary diameters were \( \approx 6 \) to 7 \( \mu \text{m} \) under all experimental conditions. Obviously, the administration of neither indomethacin nor L-NAME changed baseline Ppa or microvascular diameters when normoxic-normocapnic conditions were maintained (Table 1).

**Microvascular Diameter Changes at Hypoxia, Hypercapnic Acidosis, and Isocapnic Acidosis in the Medication-Free Group**

Introduction of hypoxia enhanced the Ppa by 4.1 mm Hg in association with a 2.7-\( \mu \text{m} \) reduction in arteriolar diameter,
Table 2. Effects of Indomethacin and L-NAME on Mean Ppa Incrementation

<table>
<thead>
<tr>
<th>Condition</th>
<th>N Group</th>
<th>I Group</th>
<th>L Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxia</td>
<td>4.1 ± 2.0</td>
<td>4.6 ± 2.4</td>
<td>11.1 ± 2.0*</td>
</tr>
<tr>
<td>Hypercapnia</td>
<td>0.7 ± 0.6</td>
<td>0.4 ± 0.4</td>
<td>1.1 ± 1.4</td>
</tr>
<tr>
<td>Isocapnic acidosis</td>
<td>1.0 ± 0.4</td>
<td>1.2 ± 1.5</td>
<td>1.2 ± 1.5</td>
</tr>
</tbody>
</table>

N group indicates no medication; I group, indomethacin administration; and L group, L-NAME administration. Numbers in parentheses indicate number of animals studied. Values are mean ± SD. Ppa increments under all experimental conditions were statistically significant; ie, there was an authentic increase in at least one group as compared with the N group. Values in groups 2–6 differ from values in the N group. N, significantly (P < .05) greater than values in the N group.

Microvascular Diameters at Hypoxia, Hypercapnic Acidosis, and Isocapnic Acidosis in the L-NAME–Treated Group

Although increments of perfusate concentrations of NO-related products before and after hypoxic stimulation attained 2.8 mmol/L in the absence of L-NAME, they were only 0.4 mmol/L when L-NAME was administered (Table 3). Qualitatively, the same trend was observed under the conditions of hypercapnia and of isocapnic acidosis (Table 3).

Microvascular Diameter Changes at Hypoxia, Hypercapnic Acidosis, and Isocapnic Acidosis in the Indomethacin-Treated Group

The perfusate concentrations of 6-keto-PGF_1α, after hypoxic stimulation in the absence of indomethacin were appreciably increased, whereas those in the presence of indomethacin were suppressed (Table 3). Qualitatively, the same trend was observed under the conditions of hypercapnia and of isocapnic acidosis (Table 3).

Addition of indomethacin did not alter the overall pressor response (Table 2) or the constrictive level of arterioles during hypercapnic acidosis but converted the venular state from dilation to constriction (Fig 1). Capillary diameter at hypercapnic acidosis was not modified by indomethacin.

The increment of Ppa and diameter changes in microvessels at isocapnic acidosis in the presence of indomethacin did not differ from the changes obtained in the agent’s absence (Table 2 and Fig 1).

Microvascular Diameters at Hypoxia, Hypercapnic Acidosis, and Isocapnic Acidosis in the L-NAME–Treated Group

Although increments of perfusate concentrations of NO-related products before and after hypoxic stimulation attained 2.8 mmol/L in the absence of L-NAME, they were only 0.4 mmol/L when L-NAME was administered (Table 3). Qualitatively, the same trend was observed under the conditions of hypercapnia and of isocapnic acidosis (Table 3).

Figure 1. Differential effects of indomethacin and L-NAME on microvascular response to hypoxia, hypercapnia, and isocapnic acidosis in the acini (mean ± SD). A, Arteriolar response. B, Venular response. Groups are as follows: N, arteriolar and venular response to a certain stimulus obtained under medication-free conditions (n = 7 for hypoxia, n = 23 for hypercapnia, and n = 11 for isocapnic acidosis); I, arteriolar and venular response during indomethacin administration (n = 6 [hypoxia], n = 7 [hypercapnia], and n = 5 [isocapnic acidosis]); L, arteriolar and venular response in the presence of L-NAME (n = 6 for hypoxia, hypercapnia, and isocapnic acidosis). *Significant (P < .05) vasoconstriction or vasodilatation. #P < .05 vs N group values. +P < .05 vs I group values.
venular nor capillary reactivity to isocapnic acidosis was changed by the presence of L-NAME.

**pH\textsubscript{i} During Hypercapnia and Isocapnic Acidosis**

pH\textsubscript{i} of HPAECs before the imposition of hypercapnic acidosis averaged 7.16±0.05 and was promptly changed immediately after the initiation of hypercapnia, attaining the plateau value of 6.83±0.01 within 1 minute (Fig 2). Baseline pH\textsubscript{i} before the introduction of HCl was 7.19±0.02, which was not different from the value obtained before hypercapnia exposure. Alterations of pH\textsubscript{i} during isocapnic acidosis were more gradual than those during hypercapnic acidosis and reached a nadir of 6.86±0.01 approximately 3 minutes later (Fig 2). The nadir level of pH\textsubscript{i} in isocapnic acidosis was comparable to that in hypercapnic acidosis. Thereafter, pH\textsubscript{i} during isocapnic acidosis slowly increased at a rate of 0.016/min and reached 7.05 fifteen minutes later. The pH\textsubscript{i} values obtained between 7 and 15 minutes after HCl administration were always more alkaliotic than those observed after hypercapnia exposure.

**Discussion**

**Critique of Methods**

To appraise the reactivity of intra-acinar microvessels to various stimuli, including hypoxia, hypercapnia, and isocapnic acidosis, we developed a real-time confocal laser scanning luminescence optical microscope, which allows precise discrimination of individual microvessels from neighbors despite close microvessel piling. Our novel method, however, has potential limitations since it was restricted to the in situ condition; ie, we used the isolated lung perfused at a constant flow rate with a buffer containing a small quantity of whole blood (Ht, 0.05). Hemodynamic aspects in a lung isolated from an animal may be rather different from those in the living animal. Furthermore, low erythrocyte densities in the circulating medium may blunt the vascular reactivity to various physiological stimuli, such as hypoxia. Shirai and colleagues\textsuperscript{7,8} have previously reported that hypoxia evokes a vasoconstriction of feline small pulmonary veins under in vivo conditions, contradicting our findings (Fig 1). In addition to the difference in animal species and venular size analyzed, one other important distinction between the present study and their studies is the method of lung preparation; the extent of microvascular constriction, especially that of the venule, may be underestimated in the isolated lung perfused with a low Ht medium.

**Hypoxia-Induced Constriction of Intra-acinar Microvessels**

Although previous histological and radiological studies\textsuperscript{3,6,21} have indicated that in intact lungs hypoxia causes vasoconstriction in pulmonary arteries (with the diameter ranging from 100 to 1000 \( \mu \)m), the question of whether much smaller intra-acinar arterioles are actually constricted when exposed to hypoxia has not been conclusively answered. Applying a monochromatic videomicroscope, Hillier et al\textsuperscript{7} demonstrated that canine pulmonary arterioles with a diameter of \( <70 \mu \)m were constricted during exposure to hypoxic gas; these findings were qualitatively consistent with those observed in the present study (Fig 1). Although the issue of whether

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**TABLE 3. Effects of Indomethacin and L-NAME on Perfusate Concentrations of 6-Keto-PGF\textsubscript{1α} and NO-Related Metabolites Before and After Various Stimuli**

<table>
<thead>
<tr>
<th></th>
<th>6-Keto-PGF\textsubscript{1α}, pg/mL</th>
<th>NO Metabolites, ( \mu )mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indomethacin (−)</td>
<td>Indomethacin (+)</td>
</tr>
<tr>
<td><strong>Hypercapnia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>309±63 (6)</td>
<td>208±71† (8)</td>
</tr>
<tr>
<td>After</td>
<td>379±51* (6)</td>
<td>173±48† (8)</td>
</tr>
<tr>
<td><strong>Hypoxia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>286±45 (9)</td>
<td>139±50† (7)</td>
</tr>
<tr>
<td>After</td>
<td>417±58* (9)</td>
<td>113±45† (7)</td>
</tr>
<tr>
<td><strong>HCl</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>313±49 (5)</td>
<td>172±96† (6)</td>
</tr>
<tr>
<td>After</td>
<td>360±54* (5)</td>
<td>111±72† (6)</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the number of samples used for analysis.

*Significantly (\( P<.05 \)) larger than the values before each stimulation. †Significantly (\( P<.05 \)) smaller than the values obtained in the absence of medication.

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![Figure 2. Transitional changes in pH\textsubscript{i} of HPAECs obtained from human large pulmonary arteries on hypercapnia and isocapnic acidosis (n=5 for both). For clarity of presentation, error bars are omitted, and only mean measurement values are shown. *Significant difference (\( P<.05 \)) between hypercapnia and isocapnic acidosis at the same time point (unpaired t test). #Significant difference (\( P<.05 \)), as a whole, between hypercapnia and isocapnic acidosis (Hotelling T\(^2\) statistic).](image-url)
intra-acinar arterioles have a significant amount of cells capable of vasoconstriction has been largely controversial.\textsuperscript{24,25} Our experimental results appear to support the findings of Davies et al.\textsuperscript{25} who showed that intra-acinar arterioles with a diameter of $>10 \, \mu m$ have appreciable quantities of cells with contractile properties.

The overall extent of the hypoxia-induced pressor response was significantly enhanced by inhibiting NOS but not by inhibiting COX (Table 2), suggesting that NO would function as an important modulator coping with excessive vasoconstriction of intact pulmonary vessels during hypoxic stimulation. These findings are in accordance with those reported by several groups of investigators.\textsuperscript{9–15,26} Our results additionally suggest that vasodilating PGs have little impact in opposition to the overall HPV occurring in intact rat lungs. These findings are consistent with those of other investigators,\textsuperscript{16–18} who have demonstrated that COX-related PGs exert no conspicuous influence on Ppa changes elicited by hypoxia in the intact lung. However, the importance of endogenous PGs in attenuating overall HPV was found in endotoxin-injured rat lungs\textsuperscript{27} as well as in canine lungs during isoflurane anesthesia.\textsuperscript{28}

Although NOS inhibition notably enhanced Ppa changes (Table 2) responding to hypoxia, it did not alter the diameter of intra-acinar microvessels (Fig 1), indicating that NO would play no significant role in modulating hypoxia-induced vasoconstriction of microvessels. Assuming that Ppa changes reflect the sum of pressor response yielded by all of the intra- and extra-acinar vasculature, our experimental findings suggest that NO is important in preventing excessive vasoconstriction of relatively large resistive vessels located mainly outside the acini during hypoxia exposure.

We found no effects of COX inhibition on either the extent of vasoconstriction within the acinar microvessels (Fig 1) or the overall pressor changes during hypoxic stimulation (Table 2), indicating that COX-related vasoactive substances play little role in modifying the reactivity of intra-acinar or extra-acinar vessels to hypoxia, at least in intact rat lungs.

**Intra-acinar Microvessel Vasodilatation Induced by CO\textsubscript{2} Molecule**

Brimioulle et al.\textsuperscript{4} found that the overall HPV in the canine lung is much greater in isocapnic acidosis than in hypercapnic acidosis. Baudouin and Evans\textsuperscript{5} have demonstrated that hypercapnic acidosis attenuates HPV in the isolated rat lung. These findings indirectly suggest pH-independent vasodilation by hypercapnia in the intact lung. However, the importance of endogenous PGs in attenuating overall HPV was found in endotoxin-injured rat lungs\textsuperscript{27} as well as in canine lungs during isoflurane anesthesia.\textsuperscript{28}

Interestingly, the venular dilatation observed at hypercapnia was converted to constriction when COX was inhibited but was not affected by NOS inhibition (Fig 1), indicating that CO\textsubscript{2}-induced dilatation of venules is substantially mediated by endogenous vasodilating PGs but not by NO-related metabolites. Convincing evidence of the important role of vasoactive PGs in hypercapnic vasodilation was also demonstrated in a study involving the newborn pig brain.\textsuperscript{32} Our experimental results showing hypercapnia-induced Ppa changes, which were not altered by NOS inhibition (Table 2), are qualitatively consistent with the findings of Dumas et al.,\textsuperscript{33} who demonstrated that inhibition of NO production did not potentiate the overall HPV under hypercapnic acidosis in the perfused rat lung. In a recent study involving mice in which the neuronal NOS was knocked out, Irikura et al.\textsuperscript{34} found that cerebrovascular dilatation by hypercapnia was reliably preserved, indicating that the NO-independent pathway plays a role in the response to hypercapnia in the brain as well.

**H\textsuperscript{+}-Mediated Vasoconstriction of Intra-acinar Microvessels**

Although several studies\textsuperscript{4,15,36} devoted their attention to possible roles of isocapnic acidosis (or alkalosis) in the pulmonary circulation, the most crucial issue in these studies is that transitional changes in pH, corresponding to changes in pHi, were not studied under conditions in which acid or base was added to the surrounding medium. For instance, Raffenstein and McMurtry\textsuperscript{35} and Farrukh et al.\textsuperscript{36} implicitly assumed that neither extracellular acidification nor alkalization significantly alters pHi, at least within 10 minutes, leading them to assume that the response of pulmonary circulation to acid or base might be principally mediated by changes in pHi. We found that pericellular acidification by HCl lowered the pH within HPAECs at a rate slower than that caused by hypercapnic acidosis but led it to the nadir level within 3 minutes (Fig 2). These findings may allow us to conclude that the pressor response within 10 minutes after acute acidification by HCl should be taken to be elicited by both pHi and pHi changes, as in the case of hypercapnic acidosis. Although we investigated pHi changes solely in HPAECs harvested from human large pulmonary arteries, these observations may qualitatively be generalized to endothelial cells and smooth muscle cells of the rat pulmonary microvasculature.
Neither isocapnic acidosis nor hypercapnic acidosis, in the absence of medication, enhanced the constriction of precapillary arterioles, whereas H⁺-elicited arteriolar constriction was evidently observed at isocapnic acidosis with NOS inhibition (Fig 1). Isocapnic acidosis with COX inhibition and hypercapnic acidosis with suppression of either COX or NOS did not restore arteriolar constriction (Fig 1). These findings suggest that arteriolar constriction on H⁺ stimulation is suppressed both by H⁺-dependent activation of NOS and by COX-evoked arteriolar dilatation, the latter of which appears to be unrelated to vasodilating substances yielded by COX or NOS. Although NOS inhibition augmented arteriolar constriction responding to H⁺, it did not increase overall pressor changes during isocapnic acidosis (Table 2), indicating that NO may not play a role in modifying vascular reactivity to excessive H⁺ in extra- and intracellular spaces.

In conclusion, microvascular tones in the acini are actively but differently modulated by O₂, CO₂, and/or H⁺ in association with or independent of COX- or NOS-related vasoactive substances. The arteriolar constriction caused by hypoxia is only negligibly modulated by COX- and NOS-related metabolites, whereas venular dilatation at hypercapnia is importantly mediated by vasodilating substances produced through the CO₂-dependent activation of COX. Although arteriolar constriction is potentially induced by increased concentrations of extracellular and/or intracellular H⁺, it is generally hidden by NO-related products yielded through H⁺-associated augmentation in NOS activity.

**References**