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

Kazuhiro Yamaguchi, Koichi Suzuki, Katsuhiko Naoki, Kazumi Nishio, Nagato Sato, Kei Takeshita, Hiroyasu Kudo, Takuya Aoki, Yukio Suzuki, Atsushi Miyata, Harukuni Tsumura

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 N⁶-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 vasodilation, 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 in 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.⁶⁻¹⁸ 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.
when exposed to alveolar hypoxia, hypercapnic acidosis, and isocapnic acidosis.

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 isolated lung was then fixed on a microscopic stage with animals in the supine position and perfused into the systemic circulation.

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% O₂ and 5% CO₂ in N₂) to the following mixtures: (1) hypoxic-normocapnic gas composed of 2% O₂ and 5% CO₂ in N₂ for HPV to be elicited (perfusate PO₂ was reduced to containing 21% O₂ and 5% CO₂, at 8 mL/s, was used as the gas between the isolated lung and the roller pump. A gas mixture adjusted to 0.05 by adding fresh blood obtained from donor rats. The (Rotor 1500N, Taitec). Krebs-Henseleit solution with 3% bovine 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 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 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 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 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 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 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 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 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 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 isolated lung was then fixed on a microscopic stage with animals in the supine position and perfused into the systemic circulation.

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Measurements of Pulmonary Microvessel 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/V; Omnichrome) with appropriate fluoresceins, our confocal system enabled us to obtain apparently instantaneous images at 1000 frames/s. The final magnifying power of our system reached ×968, with a ×40 objective, on the video screen. The resulting field of view was 105×105 μm, corresponding roughly to a diameter of a single pulmonary microvessel running beside the terminal bronchiole.3 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 Image VSG, Kodak).

To determine microvessel diameter and architecture, we added 200 μL 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 (Quandra 840A Image 1.58, Apple). To discriminate 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 PGI₂ 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 N₂ gas, and stored frozen. At assay, the dried extract was reconstituted in the assay buffer. Immunoreactive 6-keto-PGF₁α, a stable metabolite of PGI₂, 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, NO₂⁻ and NO₃⁻, in the perfusate.30 The NO₂⁻/NO₃⁻ level was spectrophotometrically determined by modifying the method of Green et al.25 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 NO₃⁻ to NO₂⁻. 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)ethyl-
enediamine in 5% phosphoric acid, and the color of the product
yielded by the diazotization reaction was measured with a spec-tro-
photometer (US501, Unisoku) at an absorbance wavelength of
540 nm.

Measurements of pH 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 μg/mL streptomycin at 37°C in a
humidified atmosphere of 95% O2/5% CO2. Endothelial cells were
grown on a 250-mm2 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 mmol/L of a fluorescent pH
indicator, BCECF (Molecular Probes), at room temperature for 30
minutes. Fluorescence signals emitted from BCECF excited at 450
and 490 nm with a xenon strobe lamp (XPS-100, Nikon) were
photographed with a high-sensitivity SIT image-processing system (Argus-50/CA II, Hamamatsu Photonics). The fluorescence
ratios and pH were linearly related, thus allowing a simple conver-
sion of BCECF fluorescence signals to corresponding pH values.

Statistical Evaluation
Statistical differences in the results obtained for different experimental
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 during hypercapnic acidosis and isocapnic acidosis were examined on the basis of the Hotelling T statistic and the unpaired t test. Values are presented as mean±SD, and P<.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 μm and did not differ from those of postcapillary venules. Baseline capillary diameters were 6 to 7 μm 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-μm reduction in arteriolar diameter,
corresponding to an ~10% decrease against the baseline value, but did not induce any changes in venular or capillary diameters (Table 2 and Fig 1).

Hypercapnic acidosis modestly increased the Ppa by 0.7 mm Hg (Table 2). Hypercapnic acidosis yielded no arteriolar constriction but caused a distinct venular dilatation (Fig 1). The venular diameter was enlarged by 2 μm, ie, an 8% increase in the venular size. Hypercapnic acidosis did not influence capillary diameters.

Isocapnic acidosis increased the Ppa by 1 mm Hg (Table 2) in association with no alteration of microvascular diameters (Fig 1).

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

The perfusate concentrations of 6-keto-PGF\(_{1\alpha}\) 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).

Administration of indomethacin did not influence the overall extent of pulmonary vasoconstriction (Table 2) or precapillary arteriolar diameter on hypoxic stimulation (Fig 1). Hypoxia did not evoke any diameter change in venules and capillaries in the presence of indomethacin (Fig 1).

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 μmol/L in the absence of L-NAME, they were only 0.4 μmol/L when L-NAME was administered (Table 3). Qualitatively, the same trend was observed for hypercapnic acidosis and isocapnic acidosis (Table 3).

Inhibition of NOS by L-NAME markedly enhanced hypoxia-induced overall pressor response of the pulmonary circulation (Table 2). The presence of L-NAME, however, exerted little influence on hypoxia-evoked diameter change in any microvessels in the acini (Fig 1).

L-NAME administration did not modify the Ppa increment (Table 2) or microvascular response to hypercapnic acidosis (Fig 1). Hypercapnic acidosis concomitant with L-NAME did not elicit arteriolar constriction and caused postcapillary venules to remain dilated by 2.1 μm (Fig 1). Capillary diameter was not altered by the presence of L-NAME at hypercapnia.

Although the extent of increase in overall Ppa at isocapnic acidosis was not modified by L-NAME administration (Table 2), the intra-acinar arterioles distinctly gained contractility in response to isocapnic acidosis; ie, the average arteriolar diameter was reduced by 1.4 μm (Fig 1). However, neither

### Table 2. Effects of Indomethacin and L-NAME on Mean Ppa Incrementation

<table>
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<tr>
<th>Condition</th>
<th>N Group</th>
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<tr>
<td>Hypoxia</td>
<td>4.1±2.0</td>
<td>4.6±2.4</td>
<td>11.1±7.0*</td>
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<tr>
<td>Hypercapnia</td>
<td>0.7±0.6</td>
<td>0.4±0.4</td>
<td>1.1±1.4</td>
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<tr>
<td>Isocapnic acidosis</td>
<td>1.0±0.4</td>
<td>1.2±1.5</td>
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*Significantly (P<.05) greater than values in the N group.

### 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 vasodilation. #P<.05 vs I group values.
Intra-acinar Microvessel Response to O₂, CO₂, and H⁺

Table 3. Effects of Indomethacin and L-NAME on Perfusate Concentrations of 6-Keto-PGF₁α and NO-Related Metabolites Before and After Various Stimuli

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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.

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.3 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.19 Shirai and colleagues3,6 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 studies3,6,21 have indicated that in intact lungs hypoxia causes vasoconstriction in pulmonary arteries (with the diameter ranging from 100 to 1000 μm), 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 al7 demonstrated that canine pulmonary arterioles with a diameter of <70 μm 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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**Figure 2.** Transitional changes in pH 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 T² statistic).

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intra-acinar arterioles have a significant amount of cells capable of vasoconstriction has been largely controversial,24,25 our experimental results appear to support the findings of Davies et al.,26 who showed that intra-acinar arterioles with a diameter of >10 μm 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.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,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 lungs27 as well as in canine lungs during isoflurane anesthesia.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 CO₂ Molecule**

Brimioulle et al29 found that the overall HPV in the canine lung is much greater in isocapnic acidosis than in hypercapnic acidosis. Baudouin and Evans30 have demonstrated that hypercapnic acidosis attenuates HPV in the isolated rat lung. These findings indirectly suggest pH-independent vasodilation by CO₂. However, no studies have directly demonstrated the portions responsible for CO₂-independent vasodilation in pulmonary circulation. Kato and Staub31 have reported that the exposure of feline lungs to hypercapnic acidosis does not constrict muscular pulmonary arteries with diameters of ≈200 μm. In contrast, Koyama and Hiramoto32 have succeeded in showing that arteries with a diameter between 150 and 250 μm on the surface of the bullfrog lung undergo significant constriction but that small veins of a similar size undergo neither constriction nor dilatation when exposed to localized hypercapnia. Shirai et al33 have reported that regional hypercapnia induces vasoconstriction of feline pulmonary vessels, including arteries and veins with diameters ranging from 100 to 600 μm. The findings of Koyama and Hiramoto29 and Shirai et al33 may indicate that small arteries located mainly outside the acini are sensitive to the constrictive effect of H⁺ rather than to the vasodilating effect induced by CO₂, though vasoactive effects of H⁺ and CO₂ on extra-acinar small veins are inconsistent. Meanwhile, we have demonstrated that exposure to hypercapnic acidosis evokes a modest increase in Ppa (Table 2) associated with a significant dilatation of venules (Fig 1). Our findings may suggest that intra-acinar microvessels with diameters of <30 μm (especially venules) acquire a higher responsiveness to CO₂-mediated vasodilation. Although the issue of whether venular walls actually have contractile cells has not been decisively answered, Joyce and colleagues30,31 have provided the evidence that venules in the rat lung possess pericytes with a contractile function.

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 CO₂-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.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,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 al34 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⁺-Mediated Vasoconstriction of Intra-acinar Microvessels**

Although several studies3,35,36 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 pHₑ, were not studied under conditions in which acid or base was added to the surrounding medium. For instance, Raffenstein and McMurtry35 and Farrukh et al36 implicitly assumed that neither extracellular acidification nor alkalinization significantly alters pHₑ, 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 pHₑ. 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 pHₑ and pHₑ changes, as in the case of hypercapnic acidosis. Although we investigated pHₑ 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⁺-elicted 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 NOS³⁶ and by CO₂-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-acinar vessels of a larger size.

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

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