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 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. However, the CO₂ molecule, per se, has been presumed to induce pulmonary vasodilation, as when Viles and Shepherd 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 investigators 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. 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.24,25 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 at a constant recirculating flow rate of 0.2 mL/s with a roller pump (Rotofont 1500N, Taitec). Krebs-Henseleit solution with 3% bovine serum albumin was used as the perfusate, in which the pH was adjusted to 7.6. After 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) connected to a small cannula whose tip was located in the trachea. The gas blown onto the lung surface were simultaneously switched with a gas containing 21% O2 and 5% CO2, at 8 mL/s, which was maintained with an ECMO (Merasilox-S, Senko) inserted into the trachea was ligated at the end-inspiratory position, and gas exchange was continuously monitored with a pressure transducer (TP-400T, 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% 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 0.3 kPa [3.8 mm Hg], 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 perfusate 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 [Sigma Chemical Co] was used to restrain a constitutive form of COX [COX-1], and the perfusate concentration of indomethacin was adjusted to 20 μmol/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 μmol/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.26 The reflected light or fluorescent emission from the sample was imaged onto a high-sensitivity CCD camera with an image intensifier (EktaPro Intensified Imager 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; Omnicrome) with appropriate fluorescent dyes, 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 bronchiolus.27 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 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 (Quadra 840AV/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.28 The NO2−/NO3− level was spectrophotometrically determined by modifying the method of Green et al.29 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

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-PGF1α = 6-ketoprostaglandin F1α
L-NAME = N-nitro-L-arginine methyl ester
NOS = NO synthase
PG = prostaglandin
Ppa = pulmonary arterial pressure
PGI2 = prostacyclin
PO2 = perfusate O2 partial pressure
PO4 = perfusate PCO2 partial pressure
Sigma Chemical Co. = Sigma Chemical Co.
Sigma = Sigma Chemical Co.
WGA = WGA

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mL of 1% sulfanilamide and 1 mL of 0.1% N-(1-naphthyl)methylenediamine 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\textsubscript{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 μg/mL streptomycin at 37°C in a humidified atmosphere of 95% O\textsubscript{2}/5% CO\textsubscript{2}. Endothelial cells were grown on a 250-mm\textsuperscript{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 (Sigma). Endothelial cell monolayers on the plate were loaded with 1% bovine serum albumin containing 2.5 μmol/L of a fluorescent pH indicator, BCECF (Molecular Probes), at room temperature for 30 minutes. BCECF-loaded cells were washed with Dulbecco’s phosphate-buffered saline (Sigma) to remove unbound dye. BCECF 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). BCECF fluorescence signals were used as a measure of pH\textsubscript{i}. 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\textsubscript{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\textsubscript{i} 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 approximately 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$_{1α}$ 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).

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 held true 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>
<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 (8)</td>
<td>4.6 ± 2.4 (7)</td>
<td>11.1 ± 7.0* (6)</td>
</tr>
<tr>
<td>Hypercapnia</td>
<td>0.7 ± 0.6 (16)</td>
<td>0.4 ± 0.4 (7)</td>
<td>1.1 ± 1.4 (6)</td>
</tr>
<tr>
<td>Isocapnic acidosis</td>
<td>1.0 ± 0.4 (7)</td>
<td>1.2 ± 1.5 (5)</td>
<td>1.2 ± 1.5 (6)</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 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

*Significantly ($P<.05$) greater than values in the N group.
venular nor capillary reactivity to isocapnic acidosis was changed by the presence of L-NAME.

**pH** During Hypercapnia and Isocapnic Acidosis

pH 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 before the introduction of HCl was 7.19 ± 0.02, which was not different from the value obtained before hypercapnia exposure. Alterations of pH 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 in isocapnic acidosis was comparable to that in hypercapnic acidosis. Thereafter, pH during isocapnic acidosis slowly increased at a rate of 0.016/min and reached 7.05 fifteen minutes later. The pH values obtained between 7 and 15 minutes after HCl administration were always more alkalotic 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 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 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 al 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
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{26} 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{2} have demonstrated that hypercapnic acidosis attenuates HPV in the isolated rat lung. These findings indirectly suggest pH-independent vasodilation by CO\textsubscript{2}. However, no studies have directly demonstrated the portions responsible for CO\textsubscript{2}-mediated vasodilation in pulmonary circulation. Kato and Staub\textsuperscript{31} have reported that the exposure of canine lungs to hypercapnic acidosis does not constrict muscular pulmonary arteries with diameters of $\approx 200$ $\mu$m. In contrast, Koyama and Hiramoto\textsuperscript{29} have succeeded in showing that arteries with a diameter between 150 and 250 $\mu$m 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 al\textsuperscript{3} have reported that regional hypercapnia induces vasoconstriction of feline pulmonary vessels, including arteries and veins with diameters ranging from 100 to 600 $\mu$m. The findings of Koyama and Hiramoto\textsuperscript{29} and Shirai et al\textsuperscript{3} 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 CO\textsubscript{2}, though vasoactive effects of $H^+$ and CO\textsubscript{2} 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 $<30$ $\mu$m (especially venules) acquire a higher responsiveness to CO\textsubscript{2}-mediated vasodilation. Although the issue of whether venular walls actually have contractile cells has not been decisively answered, Joyce and colleagues\textsuperscript{30,31} 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 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$^+$-Mediated Vasoconstriction of Intra-acinar Microvessels**

Although several studies\textsuperscript{2,15,56} have 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 $pH_r$, 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 $pH_r$, 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 $pH_r$. 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 $pH_r$ and $pH_e$ changes, as in the case of hypercapnic acidosis. Although we investigated $pH_e$ 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 hypocapnic 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 hypocapnic 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 NOS²⁰ 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-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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