Ion exchange activity in pulmonary artery smooth muscle cells: the response to hypoxia

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Received 24 January 2000; accepted in final form 25 August 2000

Madden, Jane A., Daniel E. Ray, Peter A. Keller, and Jack G. Kleinman. Ion exchange activity in pulmonary artery smooth muscle cells: the response to hypoxia. Am J Physiol Lung Cell Mol Physiol 280: L264–L271, 2001.—The purposes of this study were to determine 1) the presence of the major ion transport activities that regulate cytoplasmic pH (pHc) in cat pulmonary artery smooth muscle cells, i.e., Na+/H+ and the Na+-dependent and -independent Cl-/HCO3− exchange; 2) whether pHc changes in cells from small (SPAs) and large (LPAs) pulmonary arteries during hypoxia, and 3) whether changes in pHc are due to changes in the balance of exchange activities. Exchange activities as defined by physiological maneuvers rather than molecular identity were ascertained by fluorescence microscopy to document changes in the ratio of the pH indicator 2',7'-bis-(2-carboxyethyl)-5-((and-6)-carboxyfluorescein. Steady-state pHc was higher in LPA than in SPA normoxic smooth muscle cells. SPAs and LPAs possessed all three transport activities: in HCO3−-containing solutions, Na+/H+ exchange rather than Na+/H+ exchange set the level of pHc; in HCO3−-containing hypoxic solutions, pHc increased in SPA and decreased in LPA cells; altering the baseline pHc of a cell type to that of the other did not change the direction of the pHc response during hypoxia. The absence of Na+ prevented hypoxia-induced alkalization in SPA cells; in both cell types, inhibiting the Cl−/HCO3− exchange activities reversed the normal direction of pHc changes during hypoxia.

despite a considerable research effort to elucidate the mechanism(s) of hypoxic pulmonary vasoconstriction, the sequence of cellular events leading to the contraction of the pulmonary vascular smooth muscle are not yet fully understood. In a study of cat pulmonary arteries, our laboratory (6) found that hypoxic pulmonary vasoconstriction is a function of the small (200- to 600-μm-diameter) arteries. Smooth muscle cells isolated from these small arteries contract when they are exposed to hypoxia (7), and the free cytoplasmic calcium concentration ([Ca2+]c) also increases (12). In contrast, arteries > 800 μm in diameter, as well as their smooth muscle cells, do not contract to hypoxia, and the [Ca2+]c decreases. In a preliminary report, our laboratory (11) found that during hypoxia the cytoplasmic pH (pHc) of small pulmonary artery (SPA) smooth muscle cells increased concomitantly with the increase in [Ca2+]c. In the large pulmonary artery (LPA) cells, pHc decreased in parallel with the decrease in [Ca2+]c. Changes in pHc can alter Ca2+/calmodulin binding to modify the magnitude of myosin phosphorylation and the contractile response for a given [Ca2+]c (1). Thus changes in pHc as well as in [Ca2+]c may play a role in determining whether a pulmonary artery does or does not constrict in response to hypoxia.

pHc regulation in smooth muscle cells is accomplished through the activities of several ion transport systems (Fig. 1). Some of these transport activities are poised to acidify the cell, i.e., Na+-dependent Cl−/HCO3− exchange. Others work to alkalinize it, principally Na+/H+ and Na+-dependent Cl−/HCO3− exchange. Vasoactive agonists and cellular energy status can affect the activity and possibly the direction of ion transport activities in vascular smooth muscle (1, 5, 8, 13). For the smooth muscle cell to respond appropriately to agonists, the transport activities must be coordinated. For example, agonist stimulation of transport activity may increase the cycling of an ion between the cell and its environment, and this, in turn, may stimulate other transport activities to restore ion homeostasis (13).

How pulmonary artery smooth muscle cells regulate pHc has not been extensively studied nor has it been determined whether any of the transport activities that regulate pHc are affected by hypoxia. Na+/H+ exchange has been shown indirectly in ferret pulmonary artery (3) and directly in guinea pig main pulmonary artery smooth muscle cells (9). In the guinea pig cells, Na+/H+ exchange played an active role in pHc regulation even in HCO3−-containing buffers (9). Other than the aforementioned work by Quinn et al. (9), the presence and role of Na+-dependent and -independent Cl−/HCO3− exchange in pulmonary artery smooth muscle cells has not been studied.

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**Cell Isolation and Culture**

This study was approved by the Animal Care and Use Committee of the Zablocki VA Medical Center (Milwaukee, WI). Adult mongrel cats (2.5–4.0 kg) of either sex were anesthetized with ketamine (15 mg/kg) and pentobarbital sodium (30 mg/kg) and decapitated. The animals were exanguinated by severance of the carotid artery, and the lungs were removed under aseptic techniques and immediately placed in chilled sterile physiological saline solution (PSS). SPAs (200- to 600-μm diameter) and LPAs (>800-μm diameter) were dissected from portions of both lung lobes under sterile conditions and placed in sterile MEM plus penicillin and streptomycin (Sigma) until the dissection process was complete.

The dissected arteries were cut into 2-mm segments and placed in sterile centrifuge tubes containing 1 ml of enzyme solution. The tubes were then placed in a sterile incubator at 37°C for ~1 h. Enzyme digestion was stopped by the addition of an equal volume of smooth muscle basal medium and smooth muscle medium supplement plus penicillin and streptomycin (Sigma) until the dissection process was complete.

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**METHODS**

**Cell Acidifier**

Na+/H+ antiport (Na+/H+ exchange)

**Cell Alkalizer**

Cl-/HCO3⁻ exchange

**Fig. 1. Major ion transport mechanisms that regulate smooth muscle cytoplasmic pH (pHc) by acidification (Na⁺/Cl⁻ exchange) or alkalization (Na⁺/H⁺ and Na⁺/Cl⁻ exchange)**

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**Fluorescence Imaging**

For fluorescence imaging studies of individual smooth muscle cell pHc, the cells were loaded with 5 μl of 5 μM BCECF (Molecular Probes) for 45 min at 37°C in a humidified atmosphere of 95% air-5% CO₂. The coverslip containing the cells was placed in a specially constructed 2-ml volume chamber designed to sit on the stage of a Leitz upright microscope. Warm, gassed PSS from the reservoir was slowly perfused over the cells.

The cells were imaged at the selected wavelengths for BCECF (see pHc Calibration) with a SenSys air-cooled CCD camera (Photometrics, Tucson, AZ) and epifluorescence illumination. The epifluorescence illumination light path had an eight-position, computer-controlled filter wheel and shutter attached to the microscope. The delay between the two excitation wavelengths was on the order of 50 ms. The computer-controlled shutter was used to eliminate unnecessary specimen illumination. The microscope was linked to a Silicon Graphics SGI Indy computer equipped with RatioTool software (Inovision, Raleigh, NC).

Fluorescence measurements rely on the ratio principle, which involves selecting ion-sensitive excitation or emission wavelengths that increase or decrease on cation binding. Cells loaded with BCECF were sequentially excited at 450 and 500 nm and light emitted at 535 nm (10-nm bandwidth for all filters). The ratio of 500- to 450-nm excitation was directly proportional to pHc. Individual pulmonary artery muscle cells were selected in a field of view, and changes in their BCECF ratios were acquired every 5 s during an experiment with RatioTool software. The ratios were converted to pHc based on calibration curves (see pHc Calibration).

**pHc Calibration**

The pHc was determined from calibration curves for each cell type. The cells in the chamber were perfused sequentially with solutions (see Solutions) of pH 6.5, 7.0, and 7.5 that contained the K⁺ ionophore nigericin (10). This ionophore exchanges intracellular K⁺ for extracellular H⁺ and in the presence of a high concentration of K⁺, allows equalization of intracellular and extracellular pH. For each of the three solutions, the BCECF ratios were recorded every 5 s until the ratios were stable. The BCECF ratios obtained for each solution were plotted, and a straight line demonstrating the relationship between pH and fluorescence was fit to the curve. These curves were used to calculate pHc for the pulmonary artery smooth muscle cells used in the experiments.

**Solutions**

**Enzyme solution.** The enzyme solution contained 500 U/ml of collagenase (type II), 50 U/ml of elastase, 0.75% (wt/vol) bovine serum albumin, 4 mM ATP, and 0.25% (wt/vol) soybean trypsin inhibitor (Worthington Biochemical, Freehold, NJ).

**Pucks saline solution.** Pucks saline solution contained (in mM) 0.1 CaCl₂·2H₂O, 4.7 KCl, 1.18 KH₂PO₄, 1.19 MgSO₄·7H₂O, 120 NaCl, 0.116 Na₂HPO₄·7H₂O, 5.5 d-glucose, and 0.18% phenol red (pH 7.34).

**PSS.** PSS contained (in mM) 141 Na⁺, 4.7 K⁺, 2.5 Ca²⁺, 0.72 Mg²⁺, 124 Cl⁻, 1.7 H₂PO₄⁻, 22.5 HCO₃⁻, and 11 glucose. Solutions used for the ammonia pulse technique were prepared by replacing NaCl with 15 mM NH₄Cl. HCO₃⁻-free solution was prepared by substituting 20 mM HEPES buffer.

**Cl⁻-free solution.** This solution contained (in mM) 128 sodium gluconate, 3.3 potassium gluconate, 6 calcium glu-
conate (hemi Ca\textsuperscript{2+} salt), 1.2 KH\textsubscript{2}PO\textsubscript{4}, 0.72 MgSO\textsubscript{4}, 2.25 Na\textsubscript{2}SO\textsubscript{4}, 11 glucose, and 22.5 NaHCO\textsubscript{3}.

\textit{Na\textsuperscript{+} free solution.} This solution contained (in mM) 140 N-methyl-D-glucamine, 3.3 KCl, 1.2 KH\textsubscript{2}PO\textsubscript{4}, 0.72 MgSO\textsubscript{4}, 2.5 CaCl\textsubscript{2}, 11 glucose, and 118 HCl, adjusted to pH 7.37 with 2 M Tris buffer.

\textit{pH calibration solution.} pH calibration solution contained (in mM) 140 K\textsuperscript{+}, 2.5 CaCl\textsubscript{2}, 1 MgSO\textsubscript{4}, 50 HEPES, 10 mM nigericin, and 1 \mu M valinomycin. The solutions were titrated with 2 M Tris buffer to adjust pH to 6.5, 7.0, and 7.5.

### Exchanger Inhibitors

Exchanger inhibitors were \(3 \times 10^{-5} \text{M} \) 5-(N,N-dimethyl)-amiloride hydrochloride (DMA; Calbiochem, San Diego, CA) and 1 mM 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS; Sigma).

### Gases

Calibrated gas mixtures were pumped from weather balloons through dispersion stones into the PSS reservoir. The gas mixtures used were normoxic control (P\textsubscript{O\textsubscript{2}} 140 Torr; P\textsubscript{CO\textsubscript{2}} 37 Torr), normoxic acidosis (P\textsubscript{CO\textsubscript{2}} increased to 51 Torr), normoxic alkalosis (P\textsubscript{CO\textsubscript{2}} decreased to 21 Torr), hypoxic acidosis and hypoxic alkalosis (P\textsubscript{CO\textsubscript{2}} changed as above). The PSS from the reservoir and the cell chamber described in Fluorescence Imaging were sampled at regular intervals, and the P\textsubscript{O\textsubscript{2}}, P\textsubscript{CO\textsubscript{2}}, and pH were determined with a Corning model 278 blood gas analyzer. When the gas mixtures bubbling into the reservoir were changed during an experiment, the gas tension in the cell chamber changed within 2 min. The new values for the P\textsubscript{O\textsubscript{2}}, P\textsubscript{CO\textsubscript{2}}, and pH in the cell chamber were determined 5 min after the gas change, the point at which the BCECF ratios were stable.

### Data and Statistical Analyses

pH\textsubscript{c} values for the cells are expressed as means ± SE for \(n\) cells. To determine differences, Student’s paired and unpaired \(t\)-tests or ANOVA and Fisher’s least significant difference (LDS) test were used as appropriate. A value of \(P < 0.05\) was considered significant.

### RESULTS

#### Steady-State and Hypoxic pH\textsubscript{c}

In HCO\textsubscript{3}-containing solution under normoxic conditions, the pH\textsubscript{c} of the smooth muscle cells from SPAs and LPAs averaged 6.92 ± 0.02 and 7.02 ± 0.01, respectively (\(P < 0.05\) by unpaired Student’s \(t\)-test). When the cells were then exposed to hypoxia, the BCECF ratios stabilized within 5 min. At this point, the pH\textsubscript{c} of the SPA cells had increased to 7.02 ± 0.01, whereas in LPA cells, it had decreased to 6.96 ± 0.01 (both at \(P < 0.05\) by paired Student’s \(t\)-test). Thus with hypoxia, SPA cells become more alkaline, whereas LPA cells become more acidic (Fig. 2A).

Because the baseline normoxic pH\textsubscript{c} differed between the two cell types, we wondered if baseline pH\textsubscript{c} determined the direction of the pH\textsubscript{c} change during hypoxia and if changing the baseline pH\textsubscript{c} would alter the direction of the hypoxic change. In LPA cells, the P\textsubscript{CO\textsubscript{2}} of the bath was increased to 51 Torr to reduce the pH\textsubscript{c} closer to that of the SPA cells (6.88 ± 0.03 vs. 6.92 ± 0.01).

However, when the LPA cells were then exposed to hypoxia, the pH\textsubscript{c} still decreased to 6.81 ± 0.04 (\(P < 0.05\); Fig. 2B). In SPA cells, the P\textsubscript{CO\textsubscript{2}} of the bath was decreased to 21 Torr to raise the pH\textsubscript{c} to a level approximately that of the LPA cells (7.07 ± 0.02 vs. 7.05 ± 0.01). Again, the direction of the pH\textsubscript{c} response to hypoxia was not changed; the pH\textsubscript{c} increased to 7.14 ± 0.02 (\(P < 0.05\); Fig. 2C).

### Na\textsuperscript{+}/H\textsuperscript{+} Exchange Activity

The activity of Na\textsuperscript{+}/H\textsuperscript{+} exchange in pH\textsubscript{c} recovery from an acid load was demonstrated with the NH\textsubscript{4}Cl pulse maneuver. Brief exposure to 15 mM NH\textsubscript{4}Cl resulted in alkalization due to NH\textsubscript{4}\textsuperscript{+} diffusion into the cells. The NH\textsubscript{4}Cl was then washed out, and pH\textsubscript{c} became acidic due to NH\textsubscript{3} diffusion out of the cells together with NH\textsubscript{4}\textsuperscript{+} dissociation. pH\textsubscript{c} thus decreased to a value below the normal baseline. Recovery from this acidosis can occur by a number of different exchange processes. Figure 3 shows the typical pattern of changes in SPA pH\textsubscript{c} during an NH\textsubscript{4}Cl experiment performed in the absence of CO\textsubscript{2} and HCO\textsubscript{3} to eliminate any contribution from Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchange. This same pattern was also seen in the LPA cells.

Table 1 presents the pH\textsubscript{c} values obtained for SPA and LPA cells during this experiment. In the absence of the inhibitor DMA, pH\textsubscript{c} in SPAs and LPAs returned to baseline after NH\textsubscript{4}Cl washout in 8.36 ± 1.08 and 8.50 ± 1.42 min, respectively. With DMA, pH\textsubscript{c} recovery was minimal. These results are consistent with activation of Na\textsuperscript{+}/H\textsuperscript{+} exchange being responsible for the recovery of pH\textsubscript{c} after intracellular acidification with an NH\textsubscript{4}Cl pulse.

When the same experiments were conducted in HCO\textsubscript{3}-containing PSS, pH\textsubscript{c} also returned to baseline after NH\textsubscript{4}Cl washout (Table 1) in 3.62 ± 0.45 and 4.57 ± 0.44 min, respectively. DMA added to the washout solution did not affect pH\textsubscript{c} recovery, but if Na\textsuperscript{+} was also removed from the washout fluid, pH\textsubscript{c} did not recover in either cell type (Table 1).

Although the above experiments demonstrated the presence and activity of Na++/H\textsuperscript{+} exchange in the SPA and LPA smooth muscle cells, it did not appear that this was either solely or predominantly responsible for maintaining pH\textsubscript{c} when the cells were bathed in solutions containing CO\textsubscript{2} and HCO\textsubscript{3}. In addition to the lack of effect of DMA on pH\textsubscript{c} recovery from an acid load (Table 1), adding DMA to SPA cells in HCO\textsubscript{3}-containing PSS at steady state resulted in only a slight and insignificant acidification from 6.92 ± 0.04 to 6.87 ± 0.04. Within 2.65 ± 0.26 min, the pH\textsubscript{c} had returned to baseline (n = 8 cells). When DMA was added to LPA cells, pH\textsubscript{c} remained constant at 7.01 ± 0.02 (n = 10 cells).

Under hypoxic conditions, it did not appear that the alkalization observed in SPA cells was due to activation of the Na\textsuperscript{+}/H\textsuperscript{+} exchanger to pump H\textsuperscript{+} out of the cells. Even in the presence of DMA, exposure to hypoxia still resulted in an increase in pH\textsubscript{c} from 6.88 ±
0.03 to 7.05 ± 0.04 (data not shown; $P < 0.05$ by paired Student's $t$-test; $n = 8$ cells).

**Na$^+$-Dependent and -Independent Cl$^-$ /HCO$_3^-$ Exchange Activity**

When Cl$^-$ was removed from the medium bathing the cells, the pH$_c$ in both SPA and LPA cells increased in the manner shown in Fig. 4 and to the values shown in Table 2. This change indicated intracellular alkalinization, presumably due to Cl$^-$ efflux and HCO$_3^-$ influx or to the absence of extracellular Cl$^-$ to exchange for intracellular HCO$_3^-$. When pH$_c$ reached a new, higher steady state, extracellular Cl$^-$ was restored, and pH$_c$ decreased to baseline levels (Table 2). When DIDS, a blocker of anion transport, was present during the recovery phase, pH$_c$ remained significantly above baseline ($P < 0.05$; Table 2). If Cl$^-$ was restored but Na$^+$ was removed from the PSS, the pH$_c$ continued to decline to a level significantly below baseline ($P < 0.05$; Table 2). This suggests that the recovery from the cellular alkalosis is mediated by Na$^+$-independent Cl$^-$ /HCO$_3^-$ exchange.

Na$^+$-dependent Cl$^-$ /HCO$_3^-$ exchange helps to maintain steady-state pH$_c$ in pulmonary artery smooth muscle cells by exchanging cellular Cl$^-$ for extracellular Na$^+$ and HCO$_3^-$ (Fig. 1). When Na$^+$ was removed from the HCO$_3^-$-containing PSS bathing SPA cells, the pH$_c$ decreased from 6.97 ± 0.02 to 6.45 ± 0.03 ($P < 0.05$; $n = 7$ cells), reflecting intracellular acidification. Returning Na$^+$ to the PSS restored the pH$_c$ to baseline, even in the presence of DMA. LPA smooth muscle cells showed a similar response to Na$^+$ removal, decreasing from 7.00 ± 0.02 to 6.59 ± 0.07 ($P < 0.05$; $n = 6$ cells). However, when Na$^+$ was restored to LPA cells treated with DMA, the pH$_c$ increased above baseline to 7.84 ±
intrinsic activity of Na\(^+\) to control baseline pH\(_c\). The presence of Na\(^+\) by ANOVA and Fisher's LSD test; Fig. 5.)

In the presence of the Na\(^+\) exchange inhibitor DMA, or in Na\(^+\)-free solution plus DMA during the recovery phase. *P < 0.05 compared with control baseline pH\(_c\).

When SPA cells were exposed to hypoxia in the absence of Na\(^+\), the pH\(_c\) did not increase; rather it decreased from 6.86 \pm 0.02 to 6.68 \pm 0.02 (P < 0.05; n = 6 cells). In LPA cells, the pH\(_c\) still decreased during hypoxia from 6.66 \pm 0.08 to 6.49 \pm 0.09 (P < 0.05; n = 7 cells). That the activity of both Cl\(^-\)/HCO\(_3\)\(^-\) exchangers determines the pH\(_c\) response to hypoxia is further suggested by the data shown in Fig. 5. When these exchangers were inhibited in SPA smooth muscle cells either with HEPES-buffered PSS or by exposing the cells to DIDS in the presence of CO\(_2\) and HCO\(_3\), the pH\(_c\) decreased during hypoxia (P < 0.05 by ANOVA and Fisher's LSD test; Fig. 5A). The pH\(_c\) of similarly treated LPA cells increased in response to hypoxia (P < 0.05 by ANOVA and Fisher's LSD test; Fig. 5B). Under normoxic conditions, exposing SPA cells to DIDS resulted in a decline of 0.06 \pm 0.01 pH units (P < 0.05) but no significant change in LPA cells (data not shown).

**DISCUSSION**

The major findings of this study were that 1) in the presence of CO\(_2\) and HCO\(_3\), pH\(_c\) was higher in the smooth muscle cells of LPAs than of SPAs; 2) activation of Na\(^+\)/H\(^+\) exchange as well as both Na\(^+\)-dependent and -independent Cl\(^-\)/HCO\(_3\) exchange could be demonstrated in SPA and LPA smooth muscle cells; 3) in the presence of CO\(_2\) and HCO\(_3\), Cl\(^-\)/HCO\(_3\) exchange rather than Na\(^+\)/H\(^+\) exchange appeared to be more active in regulating pH\(_c\) under normoxic conditions; 4) under hypoxic conditions, pH\(_c\) increased in SPA cells and decreased in LPA cells; 5) altering the baseline pH\(_c\) of either cell type to a level characteristic of the other did not change the usual direction of the pH\(_c\) response during hypoxia; 6) the absence of Na\(^+\) but not the pharmacological inhibition of Na\(^+\)/H\(^+\) exchange prevented the hypoxia-induced alkalization in SPA cells; and 7) inhibiting Cl\(^-\)/HCO\(_3\) exchange activity in the two cell types reversed the normal direction of pH\(_c\) changes during hypoxia.

The finding in this study that under normoxic conditions pH\(_c\) was higher in LPA cells than in SPA cells agrees with a preliminary report from our laboratory (11). The decreased pH\(_c\) in hypoxic LPA cells is also consistent with that report and agrees with the recent study by Leach et al. (4) where pH\(_c\) decreased in large-diameter pig pulmonary arteries during hypoxia. The increased pH\(_c\) in hypoxic SPA smooth muscle cells seen in this work and in the previous study from our laboratory (11) are, to our knowledge, the only reports of this kind. The pH\(_c\) changes in both cell types during hypoxia appear to be a consistent response to hypoxia rather than a function of baseline pH\(_c\). This was suggested by the finding that altering the baseline pH\(_c\) of each type of cell to a level characteristic of the other, i.e., increasing the pH\(_c\) of the SPA cells or decreasing it in LPA cells, did not change the normal direction of the pH\(_c\) response during hypoxia.

The role of ion transport mechanisms in regulating pulmonary artery smooth muscle cell pH\(_c\) has not been...
extensively studied. However, studies done in HCO₂⁻-free medium have provided evidence for Na⁺/H⁺ exchange activity in the pulmonary vasculature. For example, in ferret main pulmonary artery smooth muscle cells, the resting pH₉ and its recovery from acidosis depended on the external Na⁺ concentration and was most likely mediated by the Na⁺/H⁺ exchanger (3). Likewise, in guinea pig main pulmonary artery smooth muscle cells, Quinn et al. (9) demonstrated that the Na⁺/H⁺ exchanger played a significant role in regulating intracellular pH under steady-state conditions. Even in the presence of HCO₃⁻, the Na⁺/H⁺ exchanger appeared to be very active in these cells. In rat aorta smooth muscle cells, Little et al. (5) found that the Na⁺/H⁺ exchanger regulated pH₉ under control conditions, but when cellular ATP was depleted, the activity of this exchanger was attenuated and the Na⁺- and HCO₃⁻-dependent mechanisms were more active.

In the present study of cat pulmonary artery smooth muscle cells, activity of the three major ion transport mechanisms, Na⁺/H⁺ and both Na⁺-dependent and -independent forms of Cl⁻/HCO₃⁻ exchange, could be demonstrated. In CO₂⁻- and HCO₃⁻-containing solutions, Na⁺/H⁺ exchange appears to be less active than Na⁺-dependent Cl⁻/HCO₃⁻ exchange both at steady-state pH₉ and during recovery from an acid load. This was evidenced by the fact that the Na⁺/H⁺ exchange inhibitor DMA had no effect on baseline pH₉ in either cell type, nor did DMA inhibit recovery from an acid load.

Putative activity of a cell-acidifying Na⁺-independent Cl⁻/HCO₃⁻ exchange was demonstrated in both cell types by withdrawing and then restoring extracellular Cl⁻. pH₉ recovery after Cl⁻ restoration did not occur in the presence of DIDS. Additionally, if Na⁺ was not present when Cl⁻ was restored, the pH₉ continued to decline. This likely reflects continued activity of the cell acidifying exchange mechanisms, i.e., Na⁺-dependent Cl⁻/HCO₃⁻ exchange and Na⁺/H⁺ exchange. To our knowledge, this is the first evidence of a role for Na⁺-independent Cl⁻/HCO₃⁻ exchange in pulmonary artery smooth muscle cells.

We investigated whether the two Cl⁻/HCO₃⁻ exchange mechanisms observed under normoxic conditions might be involved in the pH₉ changes noted during hypoxia. Our results indicate that they were. In SPA cells treated with DMA, the pH₉ still increased during hypoxia, thereby ruling out a hypoxia-induced activation of the Na⁺/H⁺ exchanger. Na⁺ removal abrogated the alkalization observed with hypoxia in SPA cells. In fact, the pH₉ decreased rather than increased in response to hypoxia. Additionally, in HCO₃⁻-free (HEPES-buffered) PSS and in DIDS plus HCO₃⁻-removal during hypoxia.
containing PSS, the pHc decreased during hypoxia in the SPA cells. These results suggest that activation of a Na+-dependent Cl-/HCO3− exchange is responsible for the increase in pHc observed with hypoxia in SPA cells. In LPA cells bathed in HCO3−-free (HEPES-buffered) PSS and in DIDS plus HCO3−-containing PSS, the pHc increased during hypoxia. However, in Na+-free, HCO3−-containing PSS, pHc decreased as usual in the hypoxic LPA cells, suggesting that the decrease was mediated, at least in part, by Na+-independent Cl-/HCO3− exchange activity.

The results of the present studies have been integrated into an overall model describing the balance of the acid-base exchange activities examined in these studies (Fig. 6). Assuming a hypothetical smooth muscle cell that contains equal numbers of functional acidifying and alkalinizing transport mechanisms, the steady-state pHc would be set at a point where the activity versus pHc curves of the two exchangers cross each other. Increasing the amount of acidifying exchange (e.g., Na+-independent Cl-/HCO3− exchange in SPA cells) would act to lower pHc. This decline in pHc would continue until stimulation of the countervailing ion transport activity, Na+-independent Cl-/HCO3− exchange, occurs. Stimulation of Na+-independent Cl-/HCO3− exchange would serve to restore the steady state but at a higher pHc than during normoxia. Similarly, an increase in the amount of Na+-independent Cl-/HCO3− exchange in LPA cells during hypoxia would have the opposite effect, leading to a decline in pHc again consistent with the results observed in these studies.

Why the pHc changes during hypoxia were reversed in SPA and LPA smooth muscle cells bathed either in HEPES-buffered or in HCO3−-buffered PSS plus DIDS is not immediately evident. However, if the scenario outlined above pertains, the data could be interpreted as follows. In SPA cells in the presence of DIDS or in the absence of CO2 and HCO3−, Na+/H+ exchange, although present, is not sufficiently active either to raise pHc under normoxic conditions or to prevent acidification due to increasing metabolic acids during hypoxia. In LPA cells in the presence of DIDS or in the absence of CO2 and HCO3−, Na+/H+ exchange may have an enhanced role in regulating pHc. During hypoxia, this transport mechanism may, in a way analogous to our argument above for Cl-/HCO3− exchange, increase in activity, thereby increasing pHc.

Differences between cell types, preparations, and animal species as they relate to the proposed mechanisms that regulate hypoxic vasoconstriction have complicated the understanding of the response. The postulated roles for Cl-/HCO3− exchange in pHc control of the cat pulmonary artery smooth muscle cells may be specific to this species. Quinn et al. (9) found that Na+/H+ exchange appeared to play a more prominent role in pH control of guinea pig pulmonary artery

Fig. 6. Proposed balance of acidifying and alkalinizing transport activities in SPA and LPA smooth muscle cells during normoxia and hypoxia.
smooth muscle cells. Therefore, without additional evidence from studies in other species and organs, it is difficult to generalize our findings. To determine the relative contributions of the acidifying and alkalinizing exchange mechanisms in different species and among various cell types, it will be necessary to study cells under physiological conditions, e.g., in the presence of \( \text{HCO}_3^- \) and \( \text{CO}_2 \), and to know whether or not the data are derived from primary or passaged cells. Complete understanding of \( \text{pH}_c \) regulation in the pulmonary vasculature will also require studying the roles of the ion exchange mechanisms in pulmonary artery endothelial cells. Evidence from Cutaia et al. (2) has shown that prolonged exposure to hypoxia decreases activity of \( \text{Na}^+/	ext{H}^+ \) exchange isoform 1 of the \( \text{Na}^+/	ext{H}^+ \) exchanger in human pulmonary artery endothelial cells, but whether or not this result is species and/or cell specific is difficult to know. Finally, investigations into the interaction between \( \text{pH}_c \) and \( [\text{Ca}^{2+}]_e \) to determine whether changes in \( \text{pH}_c \) and \( [\text{Ca}^{2+}]_e \) are linked to one another and whether this linkage occurs through changes in the activities of the known acid-base and \( \text{Ca}^{2+} \) transport mechanisms should also be performed.

This study was supported by Veterans Affairs Medical Research funds awarded to J. A. Madden and J. G. Kleinman, by National Institute of Diabetes and Digestive and Kidney Diseases funds awarded to J. G. Kleinman, and by funds from the Division of Pulmonary Medicine of the Medical College of Wisconsin.


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