Airway surface liquid pH in well-differentiated airway epithelial cell cultures and mouse trachea

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Airway surface liquid pH has been proposed to be important in the pathophysiology of cystic fibrosis, asthma, and cough. Ratio image analysis was used to measure pH in the ASL after staining with the fluorescent pH indicator 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescin (BCECF)-dextran. ASL pH in bovine airway cell cultures grown at an air-liquid interface was 6.98 ± 0.06 in the absence and 6.81 ± 0.04 in the presence of HCO₃⁻/CO₂. Steady-state ASL pH changed in parallel to changes in bath pH and was acidified by Na⁺ or Cl⁻ replacement but was not affected by the inhibitors amiloride, glibenclamide, or 4,4'-dimethylstilbene-2,2'-disulfonic acid. In response to sudden acidification or alkalinization of the ASL by ~0.4 pH units by HCl/NaOH, ASL pH recovered to its initial value at a rate of 0.035 pH units/min (−HCO₃⁻) and 0.060 pH units/min (+HCO₃⁻); the pH recovery rate was reduced by amiloride and H₂DIDS. In anesthetized mice in which the trachea was surgically exposed for measurement of BCECF-dextran fluorescence through the translucent tracheal wall, ASL pH was 7.14 ± 0.01. ASL pH was sensitive to changes in blood pH created by metabolic (HCl or NaHCO₃ infusion) or respiratory (hyperventilation, hyperventilation) mechanisms. ASL pH is thus primarily determined by basolateral fluid pH, and H⁺/OH⁻ transport between the ASL and basolateral fluid involves amiloride-sensitive Na⁺/H⁺ exchange and stilbene-sensitive Cl⁻/HCO₃⁻ exchange. The rapid response of ASL pH to changes in systemic acid-base status may contribute to airway hypersensitivity in asthma and other airway diseases.

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of the airway epithelium and the complexity of the system, it is difficult to predict a priori the value and principal determinants of ASL pH and whether ASL pH is tightly regulated.

The purpose of this study was to define the principal determinants of ASL pH. Measurements were done on well-differentiated primary cultures of bovine airway epithelial cells grown at an air-liquid interface and in the in vivo mouse trachea. The bovine airway cell culture model was chosen as a well-established system for which there exists a considerable body of data on ion transport mechanisms, including an analysis of intracellular pH regulation (21). The polarized cell culture system permitted measurements of ASL pH in response to transporter agonists/inhibitors, ion substitution, and imposed pH gradients. The mouse trachea was studied as an in vivo model that permitted the testing of transporter agonists/inhibitors as well as clinically important systemic acid-base disturbances. The data reported here establish empirically the major determinants of ASL pH and the transporting systems involved in transepithelial pH equilibration. An important unanticipated finding was the lack of a strict regulatory mechanism maintaining absolute ASL pH.

**METHODS**

**Cell culture experiments.** Well-differentiated cultures of bovine tracheal cells were grown on collagen-coated 12-mm diameter Costar snapwell inserts with polycarbonate semi-permeable membranes at an air-liquid interface at 37°C in a 5% CO2-95% air atmosphere (27). Culture medium was changed every 2–4 days. Cultures were generally used 25–30 days after plating, at which time the electrical resistance was >300 Ωcm², and the transepithelial potential difference was >20 mV. Cell inserts were mounted (cells facing upward) in a stainless steel perfusion chamber in which the underside of the insert was perfused as described previously (12). The perfusate bathed the cell basolateral surface. The cell mucosal surface contacting the ASL faced upward. The chamber was maintained at 37°C using a PDMI-2 microincubator (Harvard Apparatus) positioned on the stage of an upright epifluorescence microscope and enclosed in a 100% humidified air-5% CO2 tent maintained at 37°C. For pH measurements, the ASL was stained with the dual-excitation wavelength pH indicator BCECF-dextran (40 kDa, Molecular Probes), dispersed in a low boiling point perfluorocarbon (Fluorinert FC-72, boiling point 56°C, 3M Company), and allowed to equilibrate for 4 h to set ASL pH, at a time when pH equilibration was found to be complete. For experiments involving HCO3⁻-free conditions, the perfusate consisted of HEPES buffer (124 mM NaCl, 5.8 mM KCl, 10 mM glucose, 1 mM CaCl2, 1 mM MgCl2, and 20 mM HEPES, pH 7.4), and the apical cell surface was exposed to a 100% humidified air atmosphere. For experiments in the presence of HCO3⁻, the perfusate consisted of 120 mM NaCl, 25 mM NaHCO3, 3.3 mM KH2PO4, 0.8 mM K2HPO4, 1.2 mM MgCl2, 1.2 mM CaCl2, and 10 mM glucose, pH 7.4, and the apical surface was exposed to a humidified 5% CO2-95% air atmosphere. In some experiments, perfusate pH was adjusted to 6.0–8.0 by titration with HCl/NaOH (for HCO3⁻-free buffer) or NaH2PO4/Na2HPO4 (for HCO3⁻-containing buffer). For ion substitution experiments, perfusate Na+ was replaced by choline+ or Cl⁻ by gluconate−. In experiments involving recovery from acute ASL acidification or alkalization, 20–50 μl of a perfluorocarbon suspension of HCl/NaOH (prepared by brief sonication) was added onto the ASL to change pH by 0.4–0.5 pH units. In some experiments, transport inhibitors were added to the perfusate or to both the perfusate and the ASL as described in RESULTS.

**Measurements in mouse trachea in vivo.** Mice (25–35 g body wt) were anesthetized with ketamine (60 mg/kg body wt) and xylazine (8 mg/kg) 15 min after pretreatment with atropine (1 mg/kg intraperitoneal) to prevent secretions as discussed previously (12). A midline incision was made in the neck to expose the trachea for measurement of fluorescence through the translucent tracheal wall. Unless otherwise indicated, the ASL was stained by instillation of 5 μl of the BCECF-dextran suspension in perfluorocarbon using a microcatheter passed through a feeding needle that was introduced via the mouth. The mouse was positioned on the microscope stage for fluorescence measurements as described below. Arterial blood (0.2–0.3 ml) was sampled through a PE-10 catheter inserted into the carotid artery, and blood pH and Pco2 were measured using a blood gas analyzer (Ciba Corning Diagnostic). After completion of the measurements, mice were euthanized by an overdose of pentobarbital (150 mg/kg). Animal protocols were approved by the University of California San Francisco Committee on Animal Research.

In some experiments, amiloride (10 mg/kg of 1 mM solution) was injected intraperitoneally 30 min before anesthesia, and 2.7 μg of amiloride (dispersed in perfluorocarbon) was instilled into trachea together with BCECF-dextran. ASL pH was measured 10 min after the perfluorocarbon instillation. Mice were treated with glibenclamide by intraperitoneal injection of 0.3 ml of a 1 mM glibenclamide solution and intratracheal instillation of 4.9 μg of glibenclamide in perfluorocarbon. To create acute metabolic acidosis or alkalosis, HCl (0.5 meq H+ or NaHCO3 (0.3 meq) were injected intraperitoneally. ASL pH was measured after 20 min. To create respiratory acidosis or alkalosis, the upper trachea of anesthetized, paralyzed (pancuronium, 1 mg/kg intraperitoneal) mice was cannulated with PE-90 tubing, and mice were mechanically ventilated with room air (tidal volume 8 ml/kg, respiratory rate 90 respirations/min) using a mouse constant volume ventilator (Harvard Apparatus). Acute hyperventilation was produced by increasing the respiratory rate to 140 respirations/min for 10 min before ASL pH measurements and arterial blood gas analysis. Acute hypoventilation/hypercarbia was produced by decreasing respiratory rate to 90 respirations/min and addition of 5% CO2 (95% O2, to prevent hypoxia) to the inspired gas.

**Fluorescence microscopy.** The chamber containing the cultured cells or the mouse was positioned on the stage of a Leitz upright fluorescence microscope with a Technical Instruments coaxial-confocal attachment. Fluorescence was detected using a Nikon ×50 extra-long working distance air objective (numerical aperture 0.55, working distance 8 mm) for ratiometric measurement of pH at 440- and 490-nm excitation wavelengths and a 535-nm emission wavelength. Background fluorescence (unstained cells or trachea) was <1% of total fluorescence.
RESULTS

Cell culture experiments. The ASL of tracheal cells cultured at an air-liquid interface was stained with the pH indicator BCECF-dextran by addition of microliter quantities of a low boiling point perfluorocarbon containing the dispersed indicator. The perfluorocarbon evaporated within a few seconds, permitting the BCECF microparticles to dissolve rapidly in the ASL. The cells on the porous support were mounted in a 37°C perfusion chamber in which the basolateral surface was perfused, and the apical surface was exposed to a 100% humidified atmosphere. Figure 1A shows an in situ calibration of the ratio of BCECF fluorescence at 490- and 440-nm excitation wavelengths (F490/F440). ASL pH was set using perfusates containing high K+ and ionophores. The pKa of BCECF-dextran in the ASL was 7.05, not different from that in saline. Figure 1A also shows the averaged results from a series of measurements done in cells in the absence and presence of CO2/HCO3− (in both perfusate- and atmosphere-bathing apical surface); the individual data (right) represent results obtained from different cultures with standard errors determined from measurements done at 4–8 different locations. Averaged ASL pH was 6.98 ± 0.06 in the absence and 6.81 ± 0.04 in the presence of CO2/HCO3− (P < 0.01), lower than the perfusate pH of 7.40. Figure 1B shows images of the ASL surface recorded at excitation wavelengths of 490 nm (left) and 440 nm (middle) together with a computed ratio image (right) showing excellent pH uniformity throughout the ASL.

The sensitivity of ASL pH to changes in pH of the basolateral surface perfusate was measured in the absence and presence of CO2/HCO3−. Figure 2A shows the ASL pH at 2 h after changing perfusate pH from 7.4 to 6.0 or 8.0. ASL pH changes paralleled changes in perfusate pH. The pH changes were reversible, and cells remained viable after incubation in the acidic and alkaline media as shown by transepithelial resistance (400–800 V cm−2) and trypan blue dye exclusion. Figure

Fig. 1. Airway surface liquid (ASL) pH measurement in bovine tracheal epithelial cells using 2’7’-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF)-dextran. A: calibration of the dependence of BCECF-dextran fluorescence excitation ratio (F490/F440) as a function of pH in solution (○) and ASL (-) (left). Also shown is pH measured in the ASL in the absence (●) and presence (■) of CO2/HCO3− (SE, n = 10–14 cultures). Right: pH measured in individual cultures. Each point represents the mean ± SE of 4–8 measurements in different locations in each culture. B: ratio image analysis of ASL pH. Images are shown at 490-nm (left) and 440-nm (middle) excitation wavelengths along with pseudocolored ratio image (right).
2B shows the kinetics of ASL pH following changes in perfusate pH in the absence and presence of CO2/HCO3. The initial rates of pH change (in pH units/min) were −0.030 ± 0.003 (perfusate pH 6.0) and 0.023 ± 0.005 (pH 8.0) in the absence of CO2/HCO3 and −0.027 ± 0.003 (pH 6.0) and 0.025 ± 0.007 (pH 8.0) in the presence of CO2/HCO3. CO2/HCO3 did not significantly affect the initial rate of pH change.

The time course of ASL pH recovery was measured in response to sudden acidification or alkalization of the ASL by 0.3–0.5 pH units by addition of perfluorocarbon containing dispersed HCl or NaOH. Perfusate pH was maintained at 7.4. Measurements were done in the absence (Fig. 3A) and presence (Fig. 3B) of CO2/HCO3. The average ASL buffer capacity, computed from the decreased pH produced by addition of known molar quantities of H+ to perfusate (generally >50 mM H+ in the absence of cytoplasm), was 14 ± 3 mM H+ per pH unit because of the relatively low protein content of cytoplasm. ASL pH returned to its initial values over 5–10 min, with substantially more rapid pH equilibration in the presence of CO2/HCO3, as summarized in Fig. 3C. The approximate twofold increase in the rate of pH recovery in the presence of HCO3 suggests the involvement of an HCO3-dependent pathway in ASL pH equilibration.

The contribution of Na+-dependent transport to ASL pH regulation was studied using inhibitors and Na+ substitution. The inhibitors (1 mM each) amiloride (epithelial Na+ channel and Na+/H+ exchanger), furosemide (Na+/K+2Cl- cotransporter), omeprazole (K+/H+ exchanger), or 4,4′-dinitrostilbene-2,2′-disulfonic acid (Na+/2HCO3 cotransporter) were added to the perfusate. Amiloride and omeprazole (dispersed in perfluorocarbon) were also added to the ASL. Figure 4A shows ASL pH measured at 2 h after inhibitor addition. There was no significant effect of these compounds on steady-state ASL pH. Also shown is the acidified ASL following incubation of cells for 2 h with Na+-free medium (choline+ replacing Na+).

Kinetic studies were done to identify functionally the Na+ transporter(s) involved in restoring ASL pH in response to a transepithelial pH gradient. Figure 4B shows the time course of ASL pH in response to rapid ASL acidification by HCl addition. Amiloride caused a remarkable inhibition of ASL alkalization in the absence of CO2/HCO3, suggesting that H+OH- transport by Na+/H+ exchange is the principal mechanism of ASL pH equilibration in the absence of CO2/HCO3. Figure 4C summarizes the initial rates of ASL alkalization in response to HCl addition in the absence or presence of CO2/HCO3. There were significant inhibitions of ASL alkalization by amiloride in the absence but not in the presence of HCO3-. Alkalization was also blocked by perfusate Na+ substitution.

Similar experiments were done to investigate the role of Cl- transporters in the regulation of ASL pH. The inhibitors included H2DIDS (Cl-/HCO3- exchanger), glibenclamide, diphenylamine-2-carboxylate (DPC), 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) (Cl- channel, CFTR), and acetazolamide (carbonic anhydrase), and the CFTR agonist forskolin was tested. All compounds were added to the perfusate, and the Cl- transport inhibitors (dispersed in perfluorocarbon) were also added onto the ASL. Figure 5A shows that after a 2-h incubation, forskolin (20 μM) had no significant effect on ASL pH, whereas NPPB (200 μM), DPC (300 μM),
H$_2$DIDS (100 μM), and acetazolamide (100 μM) mildly acidified the ASL. Also shown is the acidified ASL following incubation of cells for 2 h with Cl$^-$-free perfusate (gluconate$^-$ replacing Cl$^-$). Kinetic studies were done in which the time course of ASL alkalinization was measured in response to addition of HCl to the ASL. Figure 5B shows the slowing of ASL alkalinization in the presence of NPPB and H$_2$DIDS. The averaged rates of

**Fig. 5.**

**A** shows the slowing of ASL alkalinization in the presence of NPPB and H$_2$DIDS. The averaged rates of

**Fig. 4.** Role of Na$^+$-dependent transport in ASL pH regulation. **A**: ASL pH (means ± SE, 4–6 cultures) measured after 2-h incubations with furosemide (200 μM), 4,4′-dinitrostilbene-2,2′-disulfonic acid (DNDS; 300 μM), amiloride (1 mM), omeprazole (300 μM), or after replacement of Na$^+$ by choline$^+$ (Na$^+$-free buffer). *P < 0.02 compared with control. **B**: time course of ASL pH recovery after acidification of ASL by HCl in the presence of amiloride and absence (○) or presence (●) of CO$_2$/HCO$_3^-$.

**Fig. 3.** ASL pH regulation in response to sudden ASL acidification or alkalinization. Time course of ASL pH after alkalinization by direct addition of NaOH (top) or acidification by HCl (bottom) in the absence of CO$_2$/HCO$_3^-$ (A) and in the presence of CO$_2$/HCO$_3^-$ (B). C: averaged initial rates (means ± SE) of ASL pH recovery (shown as positive values) for measurements done as in A and B for 4 sets of cultures. *P < 0.02 comparing ± CO$_2$/HCO$_3^-$. 

**Fig. 3.**

**A**: ASL pH (means ± SE, 4–6 cultures) measured after 2-h incubations with furosemide (200 μM), 4,4′-dinitrostilbene-2,2′-disulfonic acid (DNDS; 300 μM), amiloride (1 mM), omeprazole (300 μM), or after replacement of Na$^+$ by choline$^+$ (Na$^+$-free buffer). *P < 0.02 compared with control. **B**: time course of ASL pH recovery after acidification of ASL by HCl in the presence of amiloride and absence (○) or presence (●) of CO$_2$/HCO$_3^-$. C: averaged initial rates (means ± SE) of ASL pH recovery for measurements done as in B for 4 sets of cultures.
ASL pH alkalization are summarized in Fig. 5. The significant slowing of ASL pH recovery by H<sub>2</sub>DIDS suggests the involvement of Cl<sup>-</sup>-HCO<sub>3</sub><sup>-</sup> exchange.

**In vivo mouse trachea experiments.** ASL pH was measured in mouse trachea after staining the tracheal lumen with BCECF-dextran. The perfluorocarbon suspension of BCECF-dextran was introduced into the trachea using a small feeding needle that was passed through the mouth and then promptly withdrawn to permit spontaneous breathing. Fluorescence was detected through the translucent tracheal wall after surgical exposure of the trachea by a midline neck incision. Figure 6 shows images of the trachea at excitation wavelengths of 440 nm (left) and 490 nm (middle) and the computed ratio image (right), showing a quite uniform pH distribution. The average ASL pH in anesthetized, spontaneously breathing mice was 7.14 ± 0.01 (SE, n = 4 mice). Arterial blood gas analysis in these mice (room air) showed a P<sub>O2</sub> of 107 ± 23 mmHg, a P<sub>CO2</sub> of 52 ± 9, pH of 7.20 ± 0.04, and a computed HCO<sub>3</sub><sup>-</sup> of 20 ± 3 mM.

ASL pH in mouse trachea was measured in response to the transport inhibitors amiloride and glibenclamide after introduction both systemically, by intraperitoneal injection, and directly onto the tracheal mucosa by instillation in a perfluorocarbon suspension. Figure 7 summarizes ASL pH along with arterial blood pH (A) and P<sub>CO2</sub> (B). There was no significant effect of the inhibitors on ASL or blood pH. The effects of acute metabolic and respiratory acid-base disturbances were studied. As described in METHODS, metabolic acidosis and alkalosis were produced by intraperitoneal injection of HCl or NaHCO<sub>3</sub>; respiratory alkalosis and acidosis were produced in paralyzed, ventilated mice by hyperventilation or hypoventilation (with 5% CO<sub>2</sub>). Figure 7 shows that the maneuvers produced the predicted changes in blood pH and P<sub>CO2</sub>. Significant ASL acidification was produced by either HCl addition or
hypoventilation/CO₂ breathing. Although blood pH was increased comparably by NaHCO₃ addition or hyperventilation/CO₂, ASL pH was increased significantly only by acute respiratory alkalosis.

**DISCUSSION**

The purpose of this study was to characterize ASL pH using an established airway cell culture model and the in vivo mouse trachea. The complementary cell culture and in vivo systems were chosen to be able to test specific transporter agonists and inhibitors, perform ion substitution maneuvers, and examine the role of CO₂/HCO₃⁻, as well as to study the integrated physiological response of ASL pH to in vivo acid-base disturbances. The ratioable pH indicator BCECF-dextran was ideal for these measurements because of its excellent sensitivity to pH changes near pH 7.0 (7% change in fluorescence ratio for 0.1 unit pH change), permitting single measurements of ASL pH to better than 0.05 pH unit accuracy. We found that the ratio of ASL pH was determined by serosal fluid/blood pH. Although specific Na⁺ and Cl⁻ transporters were found to be involved in the transient response to imposed pH gradients across the airway epithelium, ASL pH was not tightly regulated and thus subject to potentially large variations in response to changes in systemic acid-base status.

Several lines of evidence have suggested a potentially important role for HCO₃⁻ in the regulation of ASL pH. In human and bovine airway cell cultures, Smith and Welsh (24) reported that short-circuit current was HCO₃⁻ dependent and inhibited by DPC and acetazolamide. In transfected fibroblasts, Poulsen et al. (20) characterized Na⁺-independent, HCO₃⁻-dependent pH regulation in cells expressing wild-type, but not ΔF508, CFTR. These studies suggested that CFTR might be permeable to HCO₃⁻. There is limited information on pH regulation in airway epithelial cells. In cultured human nasal epithelial cells, intracellular pH in the absence of HCO₃⁻ was shown to involve amiloride-sensitive Na⁺/H⁺ exchange (18). Poulsen and Machen (21) carried out a more extensive study of intracellular pH regulation in bovine tracheal epithelial cells. They found that in the absence of HCO₃⁻, pH is regulated by the amiloride-sensitive Na⁺/H⁺ exchanger, but in the presence of HCO₃⁻, the major pathway involved Na⁺- and Cl⁻-independent, HCO₃⁻-dependent transport, possibly the transport of HCO₃⁻ by CFTR. They also reported evidence for an H₂DIDS-inhibitable Cl⁻/HCO₃⁻ exchange. Recently, Choi et al. (4) reported that CFTR mutants associated with pancreatic insufficiency do not support HCO₃⁻ transport, suggesting a physiological role for CFTR-dependent HCO₃⁻ transport.

The cell culture studies here showed that although steady-state ASL pH is determined mainly by perfusate pH, transient responses to sudden changes in ASL or perfusate pH involved Na⁺ and Cl⁻ transporters. Ion substitution and inhibitor studies suggested the involvement of amiloride-sensitive Na⁺/H⁺ exchange and H₂DIDS-sensitive Cl⁻/HCO₃⁻ exchange. The recovery of ASL pH in response to sudden acidification of the ASL was strongly inhibited by amiloride or Na⁺ replacement in the absence of CO₂/HCO₃⁻, indicating that Na⁺/H⁺ exchange is the principal HCO₃⁻-independent route for transepithelial H⁺/OH⁻ transport. In the presence of CO₂/HCO₃⁻, steady-state ASL pH was slightly increased (compared with no CO₂/HCO₃⁻), and the recovery from changes in ASL pH was accelerated approximately twofold in an H₂DIDS-inhibitable manner. Steady-state ASL pH was mildly decreased by Cl⁻ replacement and by various Cl⁻ transport inhibitors. Activation of CFTR by forskolin did not affect ASL pH. Our results are best explained by Cl⁻/HCO₃⁻ exchange as the principal route for transepithelial H⁺/OH⁻ transport in the presence of HCO₃⁻, although a contribution of CFTR cannot be easily assessed because of the imperfect specificity of the Cl⁻ transport inhibitors and the complexity of the system. Assessment of the role of CFTR in HCO₃⁻ will require comparative measurements in CFTR-expressing vs. cystic fibrosis cells as well as single cell/membrane experiments.

ASL pH in the in vivo mouse trachea was measured using a minimally invasive procedure in which the trachea was exposed by a skin incision in the neck, and the ASL was stained with BCECF-dextran using a blunt feeding needle introduced via the mouth. Measurements were made without direct contact with the tracheal mucosa or invasion of the tracheal wall. We showed previously that measured ASL depth, salt content, and pH remained stable over time (12). Additionally, [Na⁺] and pH were not different as measured by direct dye addition through a tracheal window or by the less invasive procedure used here of dye addition.
through a feeding needle and measurement through the intact tracheal wall.

ASL pH in mouse trachea was 7.14 when blood pH was 7.2. The mild systemic acidosis in control mice is probably related to the anesthesia, which was maintained at a minimal level using ketamine/xylazine. Mice are quite susceptible to hypoventilation during anesthesia (22), which probably accounts for the slightly lower ASL pH (6.9–7.0) in our preliminary measurements in mice anesthetized using pentobarbital (12). As summarized in Fig. 7, acute systemic acid-base disturbances produced substantial changes in ASL pH. Metabolic and respiratory acidosis resulted in decreased ASL pH. Mild metabolic alkalosis created by HCO₃⁻ administration did not change ASL pH, whereas respiratory alkalosis (hyperventilation) producing comparable elevation in blood pH resulted in increased ASL pH. The principal conclusion from the mouse experiments is that ASL pH changes rapidly in response to systemic acid-base status. Because of the complex determinants of ASL pH in the in vivo model (e.g., changing PCO₂ during inspiration/expiration, differential CO₂ vs. HCO₃⁻ permeabilities), it is difficult to establish more than an empirical relationship among changes in serum HCO₃⁻, arterial blood PCO₂, and ASL pH.

The pH of ASL has been proposed to be important in the physiology of the cough reflex and airway reactivity. Wong et al. (26) reported that lowering airway pH decreased ASL pH. Metabolic and respiratory acidosis resulted in increased ASL pH. The principal conclusion from the mouse experiments is that ASL pH changes rapidly in response to systemic acid-base status. Because of the complex determinants of ASL pH in the in vivo model (e.g., changing PCO₂ during inspiration/expiration, differential CO₂ vs. HCO₃⁻ permeabilities), it is difficult to establish more than an empirical relationship among changes in serum HCO₃⁻, arterial blood PCO₂, and ASL pH.

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