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Extracellular HCO_3^- is sensed by mouse cerebral arteries: Regulation of tone by receptor protein tyrosine phosphatase γ

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Abstract

We investigate sensing and signaling mechanisms for H^+, HCO_3^- and CO_2 in basilar arteries using out-of-equilibrium solutions. Selectively varying pH_o, $[HCO_3^-]_o$, or pCO_2, we find: (a) lowering pH_o attenuates vasoconstriction and vascular smooth muscle cell (VSMC) Ca^{2+}-responses whereas raising pH_o augments vasoconstriction independently of VSMC $[Ca^{2+}]_i$, (b) lowering $[HCO_3^-]_o$ increases arterial agonist-sensitivity of tone development without affecting VSMC $[Ca^{2+}]_i$ but c) no evidence that CO_2 has direct net vasomotor effects. Receptor protein tyrosine phosphatase (RPTP)γ is transcribed in endothelial cells, and direct vasomotor effects of HCO_{3o}^- are absent in arteries from RPTPγ-knockout mice. At pH_o 7.4, selective changes in $[HCO_3^-]_o$ or pCO_2 have little effect on pH_i. At pH_o 7.1, decreased $[HCO_3^-]_o$ or increased pCO_2 causes intracellular acidification, which attenuates vasoconstriction. Under equilibrated conditions, anticontractile effects of CO_2/HCO_3^- are endothelium-dependent and absent in arteries from RPTPγ-knockout mice. With CO_2/HCO_3^- present, contractile responses to agonist-stimulation are potentiated in arteries from RPTPγ-knockout compared to wild-type mice, and this difference is larger for respiratory than metabolic acidosis. In conclusion, decreased pH_o and pH_i inhibit vasoconstriction, whereas decreased $[HCO_3^-]_o$ promotes vasoconstriction through RPTPγ-dependent changes in VSMC Ca^{2+}-sensitivity. HCO_{3o}^- serves dual roles, providing substrate for pH_i-regulating membrane transporters and modulating arterial responses to acid–base disturbances.

Keywords

Vascular biology, basic science, calcium imaging, confocal microscopy, electrophysiology, endothelium, experimental, pH, physiology, receptors, smooth muscle

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Introduction

Extracellular acid–base disturbances modify cerebral artery tone and contribute to metabolic regulation of blood flow.[1] Arterial tone is influenced by systemic acid–base disturbances, but is especially sensitive to local accumulation of acid equivalents when blood flow is insufficient to meet the metabolic demand. Although Gaskell first described the vascular response to extracellular acid–base disturbances in the late nineteenth century,[2] the cellular mechanisms linking altered metabolic state and acid–base disturbances to changes in resistance artery function are still not well understood.

CO_2 and HCO_3^- constitute the most important extracellular buffer system under physiological circumstances. However, due to the reactions $CO_2 + H_2O \leftrightarrows H_2CO_3 \leftrightarrows HCO_3^- + H^+$, the concentrations of the individual buffer components cannot be varied independently under equilibrated conditions, and their separate signaling roles in the vasculature have not thus far been resolved. In this study, we use out-of-equilibrium (OOE) CO_2/HCO_3^- solutions to

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determine the separate vasomotor effects of H^+, HCO_3^- and CO_2. The OOE technique is based on rapid mixing and delivery of solutions with different buffer compositions, and exploits the relatively slow kinetics of the reaction $CO_2 + H_2O \leftrightarrows H_2CO_3$.[3] Keeping the time delay from the point of mixing to the tissue below 100 ms, the chemical interconversion among CO_2/HCO_3^- buffer components is negligible, allowing us to control, accurately and independently, the pH, $[HCO_3^-]$, and pCO_2 of the delivered solution.[3]

Investigators have proposed several mechanisms for interactions of acid-base disturbances with vascular tone. Arguing in favor of direct effects of extracellular H^+ (H_o^+) on tone development in small arteries, acidosis inhibits active tension development both in the presence and absence of CO_2/HCO_3^-.[4] It should be noted, however, that in the relaxed state as well as during contraction, vascular smooth muscle cells (VSMCs) in mouse small arteries have substantially lower intracellular pH (pH_i) when CO_2/HCO_3^- is omitted from the extracellular solutions.[5] As a consequence, findings obtained under CO_2/HCO_3^--free conditions may not apply to physiological or pathophysiological circumstances. Some have argued that CO_2 and/or HCO_3^- directly affect vasomotor function of both systemic and pulmonary arteries,[6,7] although others have suggested that CO_2 and HCO_3^- affect artery tone only indirectly, through changes in pH.[8] Interpreting these studies is complicated, however, by the chemical reactions interlinking the CO_2/HCO_3^- buffer components. Because the chemical interconversion between H^+, HCO_3^-, and CO_2 has not always been appreciated[6] and because consequences of altering pH, pCO_2 and $[HCO_3^-]$ are likely to be mutually interdependent, firm conclusions regarding direct vascular effects of the individual CO_2/HCO_3^- buffer components cannot be drawn from the existing reports.

A number of candidate molecules involved in acid–base sensing have been suggested, including G-protein coupled receptors (OGR1, GPR4, TDAG8)[9] and ion channels (ASICs)[10] sensitive to pH_o. The activities of several intracellular enzymes (soluble adenylyl cyclase,[11] rho-kinase[12] and NO-synthase[12,13]) also depend on $[HCO_3^-]$ or pH. Additionally, the receptor protein tyrosine phosphatase (RPTP)γ has been proposed to serve as sensor for extracellular CO_2 or HCO_3^- in renal proximal tubules.[14] RPTPγ contains an extracellular domain with sequence homology to the carbonic anhydrases although the domain is predicted to lack carbonic anhydrase activity.[14,15]

Considering the complex nature of acid–base disturbances and their substantial clinical implications, in the present study we employ OOE CO_2/HCO_3^- solutions to elucidate the individual vasomotor effects of altered pH_o, $[HCO_3^-]_o$, and pCO_2, as well as the underlying signaling mechanisms. We demonstrate for the first time that HCO_{3o}^- has direct vasomotor effects mediated by changes in VSMC Ca^{2+}-sensitivity, and dependent on RPTPγ. With $[HCO_3^-]_o$ in the physiological range, RPTPγ activates endothelium-dependent vasorelaxant signaling cascades that are inhibited when $[HCO_3^-]_o$ is reduced. We also show that isolated changes in pH_o—in the presence of CO_2/HCO_3^-—have major effects on vasomotor responses, mediated in part through voltage-dependent Ca^{2+} channels. In addition, the combinations of low pH_o and high pCO_2 (respiratory acidosis) or low pH_o and low $[HCO_3^-]_o$ (metabolic acidosis) cause pH_i decreases that inhibit vasoconstriction, mediated by decreased VSMC Ca^{2+} sensitivity.

Methods

Male C57BL/6 wild-type or RPTPγ-knockout mice (>7 weeks old)—kept in the animal facilities at Aarhus and Case Western Reserve Universities—were euthanized by cervical dislocation. The RPTPγ-knockout mice were generously provided by Dr Joseph Schlessinger, Yale University, USA,[16] and back-crossed for six generations with the wild-type line. The animal protocols were approved by the Danish Animal Experiments Inspectorate (2009/562-56) and the Institutional Animal Care and Use Committee at Case Western Reserve University (2013-0172) that conforms to the Guide for the Care and use of Laboratory Animals published by the US National Institutes of Health. The reporting of the animal experiments conforms with the ARRIVE guidelines. Mouse breeding was performed by crossing heterozygous male and female mice in temperature-controlled facilities employing 12-h light/12-h dark cycles with food and drinking water *ad libitum*.

Mesenteric and basilar arteries (inner diameter approximately 200 μm) were dissected free from surrounding tissue under a stereo microscope. Whenever possible, experiments on arteries from wild-type and RPTPγ-knockout mice were performed in parallel.

Isometric myography and out-of-equilibrium solutions

For studies based solely on equilibrated solutions (i.e. membrane potential recordings, Figures 5(f) and (i) and 6), arteries were mounted on 40-μm stainless steel wires in a one-channel (320A; DMT, Denmark) or four-channel (610M; DMT) wire myograph and investigated under no-flow conditions. The equilibrated CO_2/HCO_3^--containing physiological salt solution (PSS) used for these studies was composed (in mM) of 119 NaCl, 22 $NaHCO_3$, 10 HEPES, 1.2 $MgSO_4$, 2.82 KCl, 5.5 glucose, 1.18 KH_2PO_4, 0.03 ethylenediaminetetraacetic acid (EDTA) and 1.6 $CaCl_2$. Solutions with reduced amounts of, or without, HCO_3^- were produced

by substituting $NaHCO_3$ with NaCl. Equilibrated CO_2/HCO_3^--containing solutions were aerated with a gas mixture of 5 or 10% CO_2/balance air, whereas CO_2/HCO_3^--free solutions were bubbled with air (i.e. 21% O_2 balance N_2).

For studies based fully or partly on OOE technology (i.e. Figures 1–3, 5(a–e) and Supplemental Figure 1), the protocol was similar to that described above except for the following. First, OOE solutions[3] were created in real time by rapidly mixing two precursor solutions (e.g. solutions 4a and 4b or 5a and 5b, see Supplemental Table 1) with dissimilar CO_2/HCO_3^-/pH compositions. Thus, at the instant of mixing, the CO_2/HCO_3^-/pH composition (e.g. solutions 4Mix or 5Mix) was out of equilibrium. The compositions of the precursor solutions and the instantaneous concentrations after mixing are summarized in Supplemental Table 1. With judicious choice of the "a" and "b" solutions, it is possible to design "Mix" solutions with any CO_2/HCO_3^-/pH combination in the pathophysiological range, making it possible for example to vary pCO_2, [HCO_3^-], or pH one at a time while holding the other two parameters fixed. The overall reaction $CO_2 + H_2O \leftrightarrows HCO_3^- + H^+$ is so slow that the disequilibrium state degrades only slightly over the course of several hundred milliseconds. Second, arteries were mounted on 50-μm tungsten pins in the heated (37 °C) stainless steel chamber of a custom-built myograph (CW121; DMT) that was designed to allow rapid mixing of two heated (37 °C) precursor solutions (e.g. 4a and 4b) through a nylon mesh (see Figure 1a). The transit time from the point of mixing at the mesh to the artery in the chamber was <100 ms. The precursor solutions were delivered through CO_2-impermeable Tygon® tubing at a rate of 7 mL/min via Pump 33 dual syringe pumps (Harvard Apparatus; MA, USA), and the mixed solutions were continuously removed by a vacuum pump. Solutions with elevated [K^+] were obtained by substituting NaCl with KCl; and when applied to mesenteric arteries, 100 nM of the α_1-adrenergic antagonist prazosin was added to avoid effects of norepinephrine released from depolarized nerve endings.

All solutions were heated to 37 °C and gassed vigorously for at least 15 min before pH was titrated to the indicated level. Data were acquired using a PowerLab 4/26 A/D converter and LabChart software (ADInstruments, New Zealand). Arteries were normalized to 90% of the internal diameter corresponding to a transmural pressure of 100 mmHg, as previously described.[17]

Intracellular pH and [Ca^{2+}] measurements

Ca^{2+} responses and pH_i were monitored in VSMCs of mouse mesenteric or basilar arteries using wide-field fluorescence microscopy as previously described.[5] Arteries were dye-loaded by addition of 5 μM BCECF-AM (Invitrogen, Denmark) or Fura-2-AM (Invitrogen) to the myograph bath, which has previously been shown to result in loading of VSMCs without detectable loading of endothelial cells (ECs).[4] Fluorescence data were acquired using an Olympus IX81 inverted microscope (PA, USA) equipped with a Hamamatsu EM-CCD camera (Japan) or using a Leica DM IRB inverted microscope connected to a PTI Deltascan system (Photon Technology International, NJ, USA). Data acquisition was controlled with Slidebook 5.0 (3i Technology, India) or Felix32 software (Photon Technology International). Arteries were alternately excited at 440 and 495 nm (BCECF) or at 340 and 380 nm (Fura-2) and emission light collected between 510 and 530 nm. Photodamage during BCECF excitation was minimized by reducing light exposure to the last 30 s of each experimental condition.[18] The BCECF fluorescence ratio was calibrated to pH values using the high-[K^+] nigericin method, as previously described.[19] [HCO_3^-]$_i$ was calculated from the Henderson–Hasselbalch equation based on the measured pH_i values and the assumption that CO_2 is in equilibrium across the cell membrane. Although the spectral properties of Fura-2 are pH-dependent, the K_D changes only marginally in the pH range 6.8–7.0 relevant to the current study.[20] Thus, no correction of the measured Fura-2 ratio was performed.

Resting steady-state values of force, [Ca^{2+}]$_i$, and pH_i were obtained by averaging values during the last 30 s of a 3-min exposure to the solution in question. The stimulated values were obtained by averaging values during the last 30 s of a 2.5-min exposure to the given agonist concentration or to increased [K^+]$_o$.

Ca^{2+} sensitivity

VSMC Ca^{2+} sensitivity under equilibrated conditions was investigated in basilar arteries from RPTPγ-knockout mice by a modification of a technique previously described by Mulvany and Nyborg.[21] Vessels loaded with Fura-2 as described above were first depleted of Ca^{2+} by a 10-s immersion in Ca^{2+}-free PSS containing 5 mM ethylene glycol tetraacetic acid (EGTA). Subsequently, the arteries were kept in Ca^{2+}-free PSS containing 0.1 mM EGTA and exposed three times to 10 μM U46619 for 2 min. The third stimulation under these conditions did not elicit any increase in force or Fura-2 fluorescence ratio, consistent with depletion of intracellular Ca^{2+}-stores. At this point, arteries were washed to Ca^{2+}-free PSS (no EGTA) and force and Fura-2 fluorescence were simultaneously recorded while [Ca^{2+}]$_o$ was increased step-wise up to 1.6 mM in the continuous presence of 10 μM U46619. VSMC

Ca^{2+}-sensitivity at pH_o 7.1 and 7.4 was compared based on the tension development to 30% and 60% of the maximal intracellular Ca^{2+}-response at pH_o 7.4.

β-galactosidase staining

The genetic insert disrupting RPTPγ-expression in the knockout mice contains a promoterless *LacZ* sequence allowing for β-galactosidase expression under control of the *Ptprg* promoter.[16] We visualized the pattern of *Ptprg* transcriptional activity using a β-galactosidase-based staining method previously described in detail.[22] Paraformaldehyde (PFA)-fixed, stained arteries were investigated either as whole-mount preparations or after paraffin-embedding and processing to 8-μm thick sections. Histological images were acquired using a Leica DMRE bright-field microscope (Denmark) equipped with a Leica DM300 digital camera.

Membrane potential measurements

Membrane potential was measured in VSMCs of isolated basilar arteries under equilibrated conditions using sharp electrodes, as previously described.[23]

Statistics

Data are expressed as mean±SEM. Unpaired, two-tailed Student's *t*-test was used for comparison of one variable between two groups. When the same parameter was measured under more than two conditions for the same mice, repeated-measures one-way ANOVA was employed. To evaluate the effects of two variables on the measured variable—measured multiple times for each mouse—we used repeated-measures two-way ANOVA followed by Bonferroni post-tests. Concentration–response relationships were evaluated using least-squares sigmoidal curve fits, and the derived $\log(EC_{50})$- and maximum values were compared using extra sum-of-squares *F*-tests. $P < 0.05$ was considered statistically significant; *n* equals number of mice. Sample sizes were selected based on previous studies. The investigators were not blinded to the genotype of the mice. Few arteries that did not develop stable tension were excluded from analyses. Data processing and statistical analyses were performed using Microsoft Office Excel 2010 and GraphPad Prism 5.04 software.

Results

We apply OOE CO_2/HCO_3^- solutions (Figure 1a) to small arteries in order to elucidate the individual vascular effects of H_o^+, HCO_{3o}^- and CO_2. Initially, we vary one of these parameters at a time, while keeping the other two constant at the normal physiological level (pH 7.40, 22 mM HCO_3^-, 5% CO_2). Figure 1(b) shows a typical isometric force recording obtained under these conditions.

Extracellular pH

We first evaluate the effect of selective changes in pH_o—at constant $[HCO_3^-]_o$ and pCO_2—on intracellular acid–base control in arteries from wild-type mice. In both basilar (Figure 2a) and mesenteric (Supplemental Figure 1a) arteries, isolated changes in pH_o cause parallel changes in VSMC pH_i, although the pH_i changes are of reduced magnitude compared to those in pH_o. Changes in $[HCO_3^-]_i$ also occur (Figure 2(b) and Supplemental Figure 1(b)). The degree of intracellular alkalinization caused by increasing pH_o by 0.3 is approximately three times greater than the degree of intracellular acidification induced by decreasing pH_o by 0.3 (Figure 2(a) and Supplemental Figure 1(a)), suggesting that VSMCs are better guarded against extracellular acidosis than alkalosis. The patterns of pH_i (Figure 2(a) and Supplemental Figure 1(a)) and $[HCO_3^-]_i$ (Figure 2(b) and Supplemental Figure 1(b)) changes are very similar in resting arteries and arteries constricted with vasocontractile agonists (norepinephrine or thromboxane analogue U46619).

Decreasing pH_o from 7.4 to 7.1 reduces U46619-induced tension development in basilar arteries (Figure 2c) and, correspondingly, attenuates norepinephrine-induced tension development in mesenteric arteries (Supplemental Figure 1c). Raising pH_o to 7.7 enhances U46619-induced tension development in basilar arteries (Figure 2c), whereas norepinephrine-induced tension development is unaffected in mesenteric arteries (Supplemental Figure 1c). We address panels (d), (e), and (g) in the following sections. In both basilar (Figure 2f) and mesenteric (Supplemental Figure 1f) arteries, the Ca_i^{2+} responses are smaller at pH_o 7.1 than 7.4, suggesting that the reduced tension development at low pH_o is at least in part explained by the lower $[Ca^{2+}]_i$, reflecting altered VSMC Ca^{2+}-handling. This interpretation is consistent with previous findings that extracellular acidosis inhibits voltage-dependent Ca^{2+}-channels.[24] It is also reinforced by the finding that, in both basilar (Figure 2(h) and (i)) and mesenteric (Supplemental Figure 1(h) and (i)) arteries, selectively decreasing pH_o from 7.4 to 7.1 attenuates contractions and Ca_i^{2+} responses induced by high $[K^+]_o$-mediated depolarization. Agonist-induced VSMC Ca^{2+} responses in both basilar (Figure 2f) and mesenteric (Supplemental Figure 1f) arteries are unaffected by a selective increase in pH_o from 7.4 to 7.7 under OOE conditions. Selectively increasing pH_o from 7.4 to 7.7 also does not affect

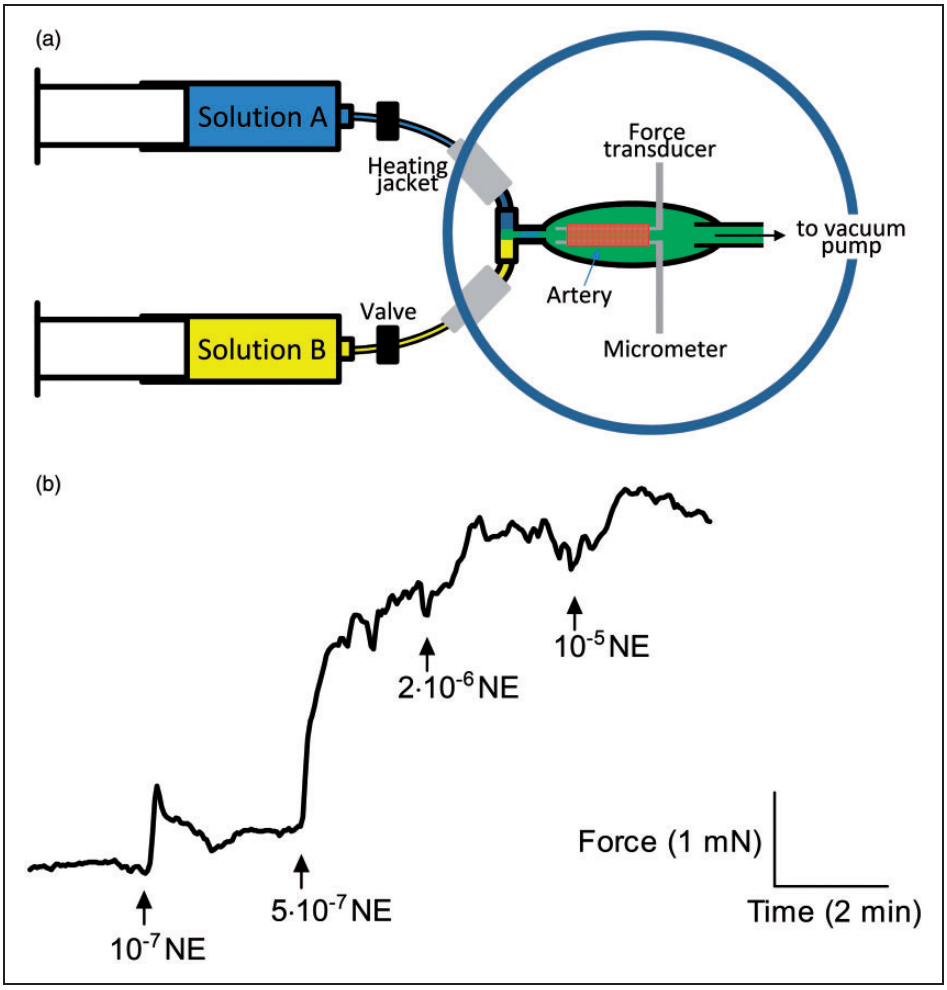

Figure 1. Application of OOE CO_2/HCO_3^- solutions to small-artery myography. (a) Schematic showing generation of OOE solutions by rapid mixing and delivery of differently composed CO_2/HCO_3^- solutions. The time delay from the point of mixing to the artery is <100 ms. See Supplemental Methods for details. (b) Force recordings from a mesenteric artery exposed to increasing concentrations of norepinephrine (NE) under OOE conditions (pH_o=7.7, $[HCO_3^-]_o$=22 mM, CO_2=5%).

tension development or VSMC Ca^{2+} responses induced by high $[K^+]_o$-mediated depolarization of basilar (Figure 2(h) and (i)) or mesenteric (Supplemental Figure 1(h) and (i)) arteries. Note that, for experiments on mesenteric arteries, we include the α_1-adrenergic inhibitor prazosin (100 nM) during the high $[K^+]_o$-induced depolarization to inhibit post-synaptic effects of the norepinephrine released from perivascular sympathetic nerves.

In further support of the notion that a selective decrease in pH_o inhibits the $[Ca^{2+}]_i$ rise in basilar arteries by inhibiting depolarization-induced Ca^{2+}-influx rather than membrane excitability, we see no effect ($n=5$; $P=0.98$, repeated measures one-way ANOVA) of equilibrated extracellular acidosis on the resting membrane potential, which is -66.6 ± 2.3 mV under control conditions (i.e. pH_o 7.40, 22 mM HCO_{3o}^-, 5% CO_2), -66.7 ± 2.5 mV during metabolic acidosis (i.e. pH_o 7.1, 11 mM HCO_{3o}^-, 5% CO_2) and -67.8 ± 6.8 mV during respiratory acidosis (i.e. pH_o 7.1, 22 mM HCO_{3o}^-, 10% CO_2).

Extracellular HCO_3^-

Selective changes in $[HCO_3^-]_o$—at a constant pH_o of 7.4 and CO_2 of 5%—have only minor effects on VSMC pH_i and $[HCO_3^-]_i$ (Figure 2(a) and (b) and Supplemental Figure 1(a) and (b)). Despite these minor effects on intracellular acid-base balance, reducing $[HCO_3^-]_o$ substantially increases the sensitivity of basilar arteries to U46619 with respect to tension development (Figure 2d), and has a comparable though considerably smaller effect on agonist sensitivity of mesenteric arteries (Supplemental Figure 1d). These findings strongly support the hypothesis that HCO_{3o}^- directly affects tension development in basilar arteries.

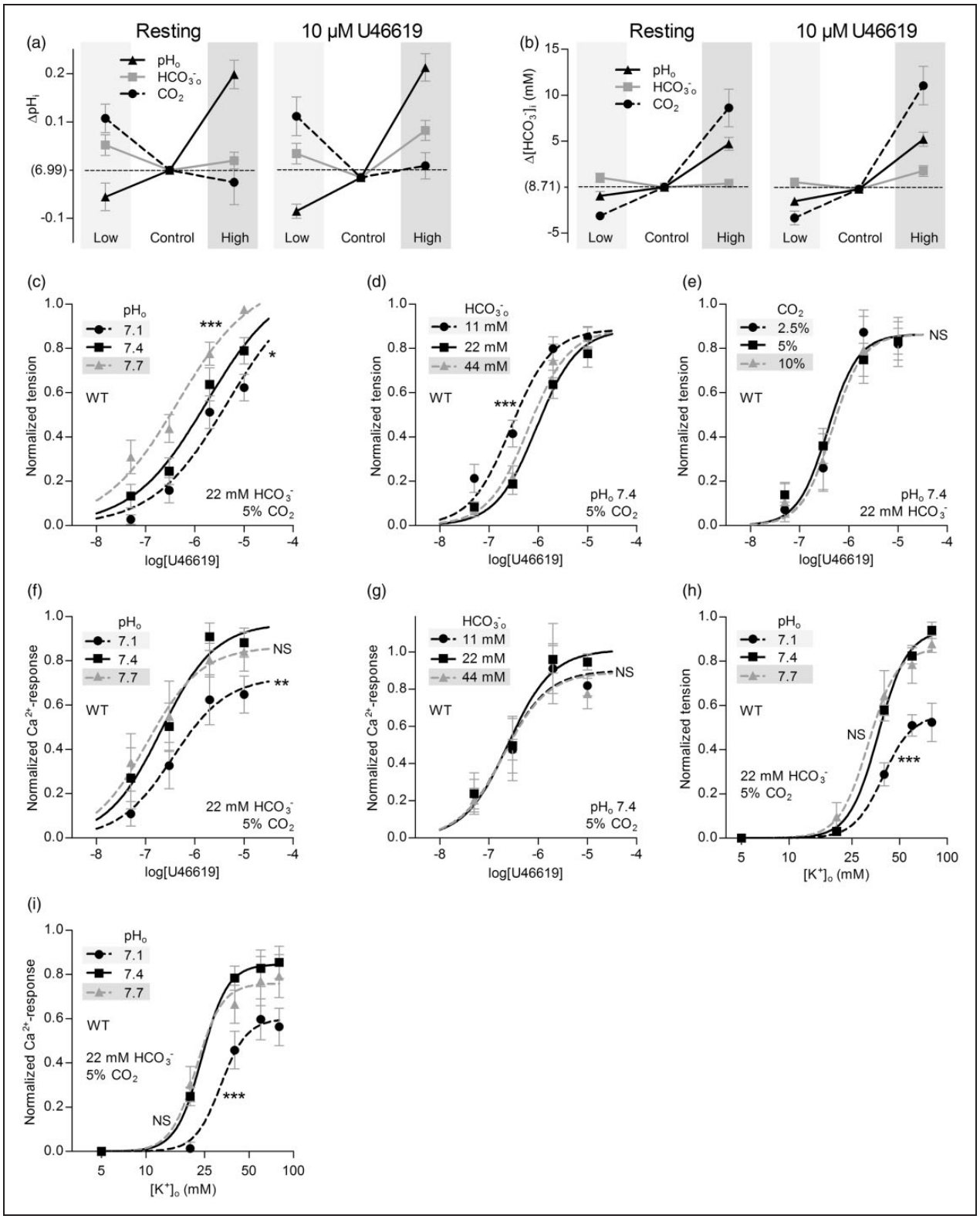

Figure 2. Selective changes in $[HCO_3^-]_o$ or pH_o—but not pCO_2—directly modulate basilar artery tone under OOE conditions. (a) Effects of selectively varying pH_o, $[HCO_3^-]_o$ or pCO_2 (maintaining other two at control levels) on VSMC pH_i in resting basilar arteries (left panel) or basilar arteries contracted by 10 μM U46619 (right panel). Under "Control" conditions, CO_2 is 5%, pH_o 7.4, and $[HCO_3^-]_o$ 22 mM. Compared to "Control", "Low" refers to selective changes in CO_2 to 2.5%, $[HCO_3^-]_o$ to 11 mM or pH_o to 7.1, and "High" refers to selective changes in CO_2 to 10%, $[HCO_3^-]_o$ to 44 mM or pH_o to 7.7. Arteries are from wild-type mice ($n = 6$). (b) Effects of selectively varying pH_o, $[HCO_3^-]_o$ or pCO_2 (maintaining other two at control levels) on VSMC calculated $[HCO_3^-]_i$ in resting

(Continued)

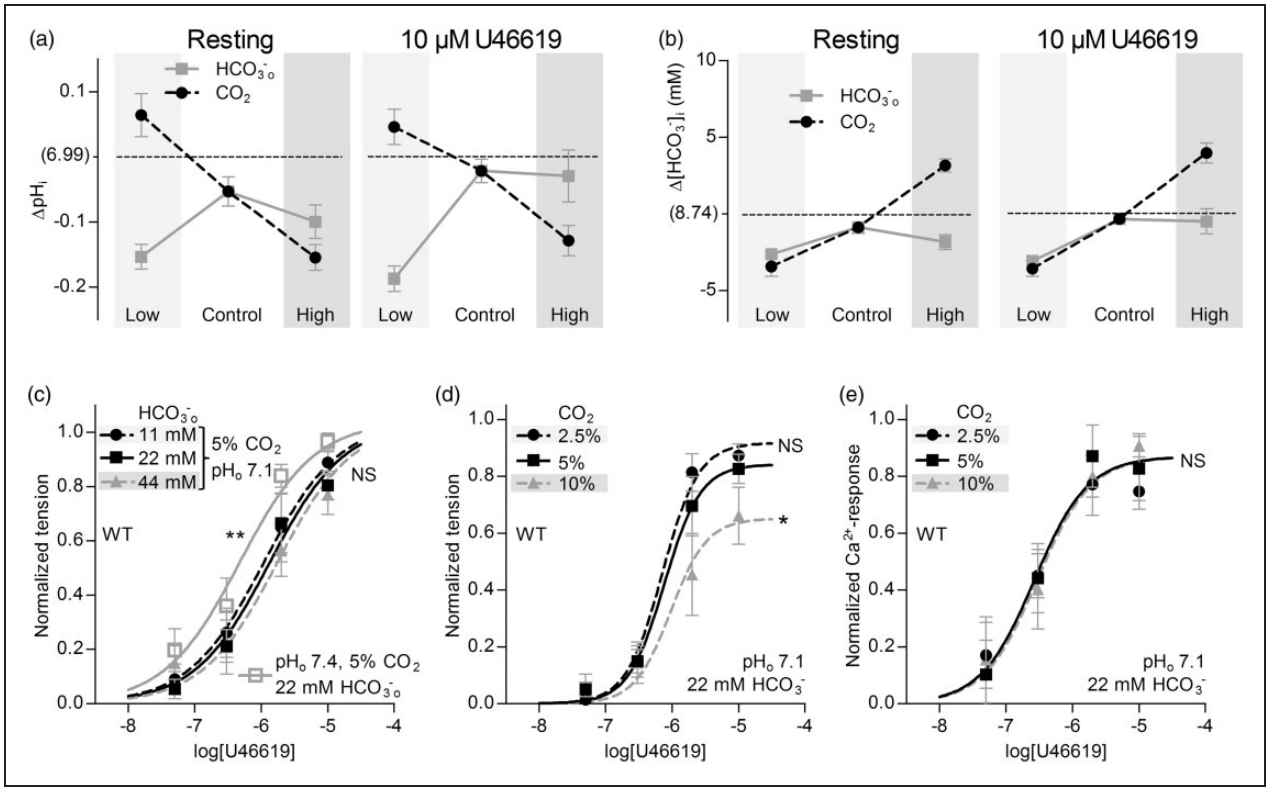

Figure 3. Increasing pCO_2 at low pH_o (pH 7.1) attenuates tension production of basilar arteries under OOE conditions. (a) Effects of selectively varying $[HCO_3^-]_o$ or pCO_2 (maintaining the other at its control level, with pH_o fixed at 7.1) on VSMC pH_i in resting basilar arteries (left panel) or basilar arteries contracted by 10 µM U46619 (right panel). "Low" and "High" refer to half and twice the concentration, respectively, of CO_2 and HCO_3^- compared to "Control" levels ($[HCO_3^-]_o$=22 mM, CO_2=5%). The reference pH_i value on the y-axis, "(6.99)", corresponds to control levels of all three parameters (pH_o=7.4, $[HCO_3^-]_o$=22 mM, CO_2=5%) in the same arteries from wild-type mice ($n = 6$). (b) Effects of selectively varying $[HCO_3^-]_o$ or pCO_2 (maintaining the other at its control level, with pH_o fixed at 7.1) on VSMC calculated $[HCO_3^-]_i$ in resting basilar arteries (left panel) or basilar arteries contracted by 10 µM U46619 (right panel). The reference $[HCO_3^-]_i$ value on the *y*-axis, "(8.74 mM)", corresponds to control levels of all three parameters (pH_o=7.4, $[HCO_3^-]_o$=22 mM, CO_2=5%) in the same arteries from wild-type mice ($n = 6$). (c, d) Effects of selectively varying $[HCO_3^-]_o$ or pCO_2 (maintaining the other at its control level, with pH_o fixed at 7.1) on U46619-induced tension development in basilar arteries from wild-type mice ($n = 6$ in panel (c), $n = 11$ in panel (d)). For comparison, in panel (c), we show the reference tension response (solid gray curve) in the same arteries, under conditions that correspond to control levels of all three parameters (pH_o=7.4, $[HCO_3^-]_o$=22 mM, CO_2=5%). (e) Effects of selectively varying pCO_2 (fixed pH_o=7.1, $[HCO_3^-]_o$=22 mM) on U46619-induced VSMC Ca^{2+} responses in basilar arteries from wild-type mice ($n = 5$). The curves in panel (c) through (e) are the results of least-squares fits to sigmoidal functions, and we compare them using extra sum-of-squares *F*-tests. *$P < 0.05$, **$P < 0.01$, NS: not significantly different *vs*. control acidic conditions (pH_o=7.1, $[HCO_3^-]_o$=22 mM, CO_2=5%).

Figure 2. Continue.

basilar arteries (left panel) or basilar arteries contracted by 10 µM U46619 (right panel). Arteries are from wild-type mice ($n = 6$). (c-e) Effects of selectively varying pH_o, $[HCO_3^-]_o$, or pCO_2 (maintaining other two at control levels) on U46619-induced tension development in basilar arteries from wild-type mice ($n = 12$ or 13). (f, g) Effects of selectively varying pH_o or $[HCO_3^-]_o$ (maintaining unvaried parameters at control levels) on U46619-induced VSMC Ca^{2+}-responses in basilar arteries from wild-type mice ($n = 6$ for both). (h) Effects of selective changes in pH_o ($[HCO_3^-]_o$=22 mM, CO_2=5%) on depolarization-induced tension development of basilar arteries from wild-type mice ($n = 8$). We induce depolarization by raising $[K^+]_o$. (i) Effects of selective changes in pH_o ($[HCO_3^-]_o$=22 mM, CO_2=5%) on depolarization-induced VSMC Ca^{2+} responses in basilar arteries from wild-type mice ($n = 7$). The curves in panels (c) through (i) are the results of least-squares fits to sigmoidal functions, and we compare them using extra sum-of-squares *F*-tests. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NS: not significantly different *vs*. control conditions (pH_o=7.4, $[HCO_3^-]_o$=22 mM, CO_2=5%).

Note that—under our experimental conditions—this sensitivity is in the low-physiological range: reducing $[HCO_3^-]_o$ from 22 to 11 mM paradoxically causes a considerable increase in the agonist-sensitivity of the basilar arteries, whereas an increase in $[HCO_3^-]_o$ from 22 to 44 mM does not affect agonist-induced tension development (Figure 2(d) and Supplemental Figure 1(d)). In contrast to the changes in artery tension development, we observe no effect of changing $[HCO_3^-]_o$ on the VSMC Ca^{2+} responses in basilar (Figure 2g) or mesenteric (Supplemental Figure 1g) arteries, suggesting that low $[HCO_3^-]_o$ modifies artery tone through changes in VSMC Ca^{2+} sensitivity.

CO_2

A selective decrease in pCO_2—at a constant pH_o of 7.4 and $[HCO_3^-]_o$ of 22 mM—alkalinizes VSMCs of mesenteric and basilar arteries, both in the resting and the contracted state (Figure 2(a) and Supplemental Figure 1(a)). In contrast, an isolated increase in pCO_2 has no significant effect on VSMC pH_i under either of these conditions (Figure 2(a) and Supplemental Figure 1(a)). As expected, increased levels of pCO_2 cause VSMC $[HCO_3^-]_i$ to increase considerably, whereas reduced levels of pCO_2 cause $[HCO_3^-]_i$ to decrease (Figure 2(b) and Supplemental Figure 1(b)).

Contrary to expectations, selective changes in pCO_2—at control levels of $[HCO_3^-]_o$ and pH_o—have no net effect on active tension development in either basilar (Figure 2e) or mesenteric (Supplemental Figure 1e) arteries.

CO_2 and extracellular HCO_3^- in basilar arteries at low extracellular pH

Acid–base disturbances in an *in vivo*, equilibrated system produce combined changes in at least two of the following: pH_o, $[HCO_3^-]_o$ and pCO_2. To determine whether the above-described cellular responses to changes in individual CO_2/HCO_3^- buffer components are interdependent, we next investigate the consequences of selectively changing $[HCO_3^-]_o$ or pCO_2 under acidic OOE conditions. Altering $[HCO_3^-]_o$ or pCO_2 at pH_o 7.1 produces intracellular acid–base responses that differ substantially from those observed at pH_o 7.4: during extracellular acidosis, VSMCs regulate pH_i less effectively in response to an increase in pCO_2 or a decrease in $[HCO_3^-]_o$ (Figure 3a). Moreover, at pH_o 7.1, VSMC $[HCO_3^-]_i$ levels (Figure 3b) are generally lower than at pH_o 7.4 (Figure 2b). These results are consistent with previous findings from other cell types showing that low pH_o inhibits cellular net acid extrusion.[25]

When selectively varying $[HCO_3^-]_o$ at pH_o 7.1, we observe no net effect on tension development of basilar arteries (Figure 3c). Although a selective decrease in pCO_2 also has no effect on artery tone at pH_o 7.1 (Figure 3d), a selective increase in pCO_2 lowers tension development (Figure 3d) with no effect on VSMC Ca^{2+} dynamics (Figure 3e). The inhibitory effect of intracellular acidification on VSMC Ca^{2+} sensitivity[5,12,18,26] likely contributes to the lower tension development observed at pH_o 7.1 compared to 7.4 in the presence of low $[HCO_3^-]_o$ (Figure 3(c) vs. Figure 2(d)) or high pCO_2 (Figure 3(d) vs. Figure 2(e)).

RPTPγ expression

RPTPγ, encoded by *Ptprg*, has been proposed to serve as extracellular CO_2/HCO_3^- sensor in renal proximal tubules.[14] Here, we study the pattern of transcriptional activity of *Ptprg* in vascular tissue of RPTPγ-knockout mice containing a gene trap insertion that brings the *LacZ* gene, encoding β-galactosidase, under control of the *Ptprg* promoter.[16] In these mice, histochemical staining for β-galactosidase activity is a reporter for *Ptprg* transcriptional activity.[22] We find that basilar arteries actively transcribe *Ptprg* (Figure 4(a) and (b)), whereas the transcriptional activity in mesenteric arteries is below detection limits (Supplemental Figure 2(a) to (d)). The β-galactosidase staining in basilar arteries is particularly prominent in ECs (Figure 4(c) and (d)).

Role of RPTPγ in basilar arteries under out-of-equilibrium conditions

Using basilar arteries from RPTPγ-knockout mice, we now study the functional importance of RPTPγ for the response to changes in $[HCO_3^-]_o$. Although U46619-induced tension in arteries from RPTPγ-knockout mice remains sensitive to selective changes in pH_o (Figure 5a), neither U46619-induced tension (Figure 5b) nor U46619-induced VSMC Ca^{2+} responses (Figure 5c) are sensitive to selective changes in $[HCO_3^-]_o$ at pH_o 7.4. At pH_o 7.1, reducing $[HCO_3^-]_o$ to 11 mM decreases the U46619 sensitivity of tension development in basilar arteries from RPTPγ-knockout mice (Figure 5d), without affecting the Ca_i^{2+} response (Figure 5e). This finding further supports the conclusion that at low pH_o, reducing $[HCO_3^-]_o$ causes a fall in VSMC pH_i (Figure 3a) that in turn reduces VSMC Ca^{2+}-sensitivity.

Role of RPTPγ for HCO_3^- sensing in basilar arteries under equilibrated conditions

To further support the role of RPTPγ in HCO_{3o}^- sensing, we study the contractile function of basilar arteries from RPTPγ-knockout mice under equilibrated

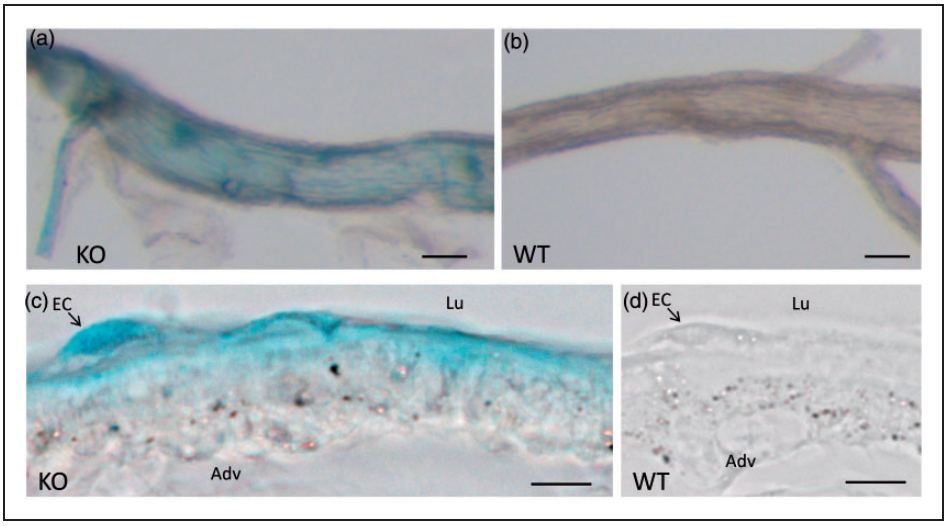

Figure 4. *Ptprg* transcriptional activity is prominent in ECs of basilar arteries. (a, b) Basilar arteries from an RPTPγ-knockout (KO, panel (a)) and a wild-type (WT, panel (b)) mouse, stained histochemically for β-galactosidase activity. Each image is representative of five experiments. (c, d) Histological sections (8 μm thick) of basilar arteries from an RPTPγ-knockout and a wild-type mouse, stained histochemically for β-galactosidase activity. Each image is representative of three experiments. The size bars in panels (a) and (b) represent 100 μm, in (c) and (d) 10 μm. Lu indicates lumen, Adv indicates adventitia and EC indicates endothelial cell.

conditions (Figure 5(f) to (i)). Omitting CO_2/HCO_3^- from the bath solution—at a constant pH_o of 7.4—increases the contractile response to U46619 in basilar arteries from wild-type mice (Figure 5f). This net enhancement of contraction in response to omission of CO_2/HCO_3^- is completely abolished in basilar arteries from RPTPγ-knockout mice (Figure 5g).

Role of endothelium-dependent relaxation for RPTPγ-mediated vasomotor effects

Considering the prominent promoter activity for RPTPγ in the endothelium of basilar arteries (Figure 4), we next investigate the role of endothelium-derived vasorelaxant influences for the HCO_{3o}^--dependent vasomotor effects of RPTPγ. We eliminate the relaxant influence of the endothelium by inhibiting NO synthesis (with 100 μM NO-synthase inhibitor L-NAME), prostacyclin production (with 3 μM cyclooxygenase inhibitor indomethacin) and endothelium-derived hyperpolarizations (EDH; with 50 nM apamin and 1 μM TRAM-34 to inhibit small- and intermediate-conductance Ca^{2+}-activated K^+-channels, respectively). To avoid development of increased resting tone after inhibition of endothelium-dependent vasorelaxation, we deliver a similar exogenous [NO] level to basilar arteries from wild-type and RPTPγ-knockout mice by incubating them with 600 nM of the NO-donor S-nitro-N-acetyl-DL-penicillamine (SNAP). We find that, following pharmacological inhibition of endothelium-dependent vasorelaxation, U46619-induced vasoconstriction of basilar arteries from wild-type and RPTPγ-knockout mice is similar in the presence and absence of CO_2/HCO_3^- (Figure 5(h) and (i)). Together, the experiments performed under equilibrated conditions support that RPTPγ provides a mechanism for endothelium-dependent, HCO_{3o}^--sensitive regulation of cerebral artery tone.

Role of RPTPγ in basilar arteries under metabolic and respiratory acidosis

To further investigate the role of RPTPγ under physiological and pathophysiological circumstances, we next compare basilar arteries from RPTPγ-knockout and wild-type mice under control and acidic conditions (Figure 6). During control conditions (i.e. pH_o 7.4, 22 mM HCO_{3o}^-, 5% CO_2), U46619 causes basilar arteries from RPTPγ-knockout mice to develop more tone than those from wild-type mice (Figure 6a). This effect persists under conditions of extracellular acidosis but is more pronounced during respiratory (i.e. pH_o 7.1, 22 mM HCO_{3o}^-, 10% CO_2; Figure 6b) than metabolic (i.e. pH_o 7.1, 11 mM HCO_{3o}^-, 5% CO_2; Figure 6d) acidosis. These findings confirm that RPTPγ activates vasorelaxant signaling mechanisms in basilar arteries and are consistent with the conclusion that RPTPγ is required for sensing of $[HCO_3^-]_o$: under physiological conditions, RPTPγ has a vasorelaxant effect that wanes under low-$[HCO_3^-]_o$ conditions. Looking at the

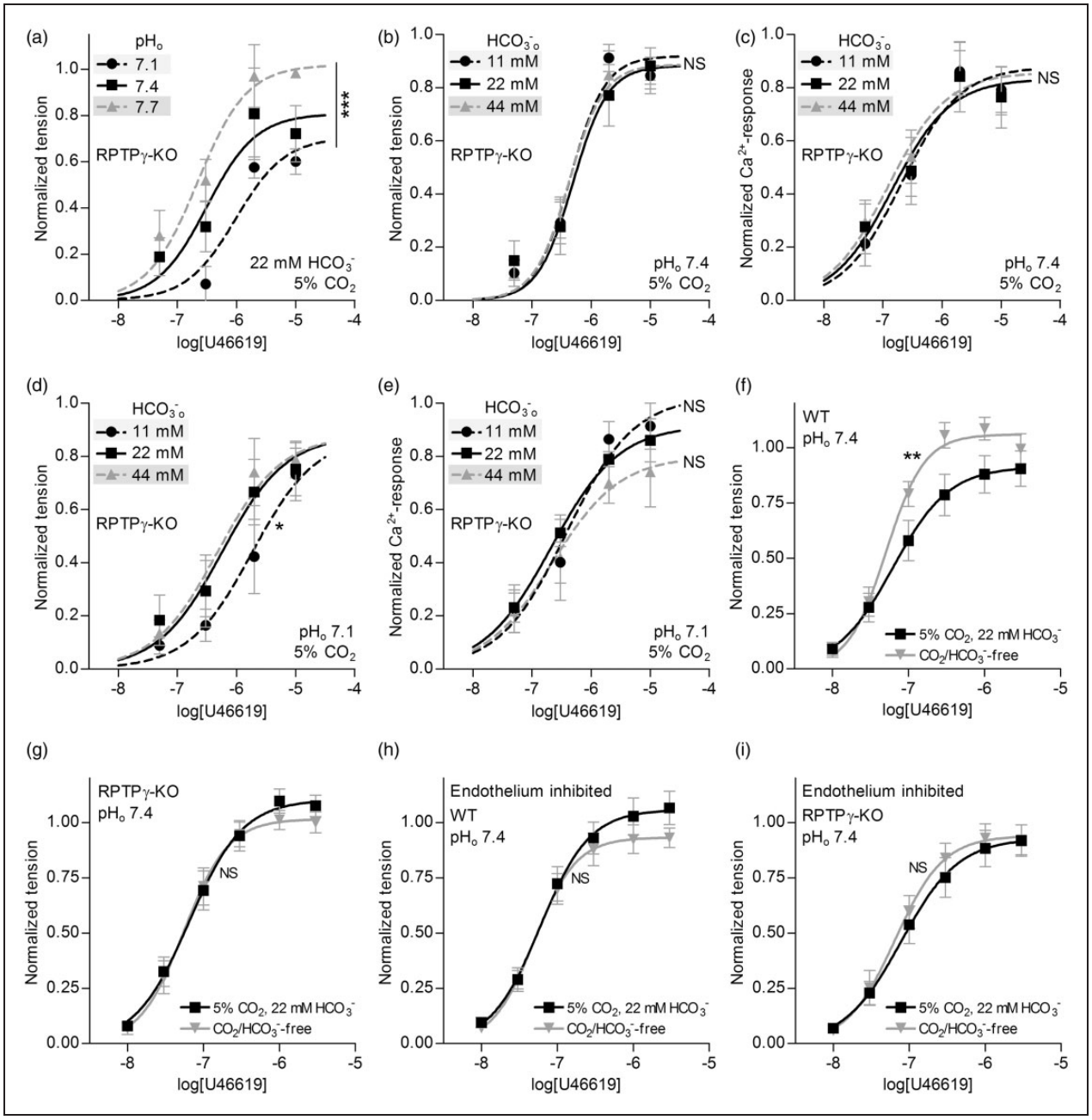

Figure 5. Basilar arteries from RPTPγ-knockout mice are insensitive to changes in $[HCO_3^-]_o$. (a, b) Effects of selectively varying pH_o or $[HCO_3^-]_o$ (maintaining the other at its control level, with CO_2 fixed at 5%) on U46619-induced tension development in basilar arteries from RPTPγ-knockout mice ($n = 4$ in panel (a), $n = 8$ in panel (b)). (c) Effects of selectively varying $[HCO_3^-]_o$ (maintaining pH_o at 7.4, CO_2 at 5%) on U46619-induced VSMC Ca^{2+}-responses in basilar arteries from RPTPγ-knockout mice ($n = 8$). (d) Effects of selectively varying $[HCO_3^-]_o$ at low pH_o (maintaining pH_o at 7.1, CO_2 at 5%) on U46619-induced tension development of basilar arteries from RPTPγ-knockout mice ($n = 6$). (e) Effects of selectively varying $[HCO_3^-]_o$ at low pH_o (maintaining pH_o at 7.1, CO_2 at 5%) on U46619-induced VSMC Ca^{2+}-responses in basilar arteries from RPTPγ-knockout mice ($n = 4$). (f-i). Effects of omitting CO_2/HCO_3^- on U46619-induced tension development at constant pH_o of 7.4. We perform experiments with basilar arteries from wild-type (WT, panels (f) and (h)) and RPTPγ-knockout (KO, panels (g) and (i)) mice. In panel (h) and (i), we inhibit vasorelaxant effects of the endothelium by incubation with 100 μM L-NAME, 3 μM indomethacin, 50 nM apamin and 1 μM TRAM-34. To avoid development of resting tone after inhibition of endothelium-dependent vasorelaxation, we add 600 nM SNAP to achieve an equal [NO] in the tested arteries. The curves are the results of least-squares fits to sigmoidal functions, and we compare them using extra sum-of-squares *F*-tests. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, NS: not significantly different *vs.* control conditions (panels (a–c) and (f–i): pH_o=7.4, $[HCO_3^-]_o$=22 mM, CO_2=5%) or standard acidic conditions (panels (d) and (e): pH_o=7.1, $[HCO_3^-]_o$=22 mM, CO_2=5%).

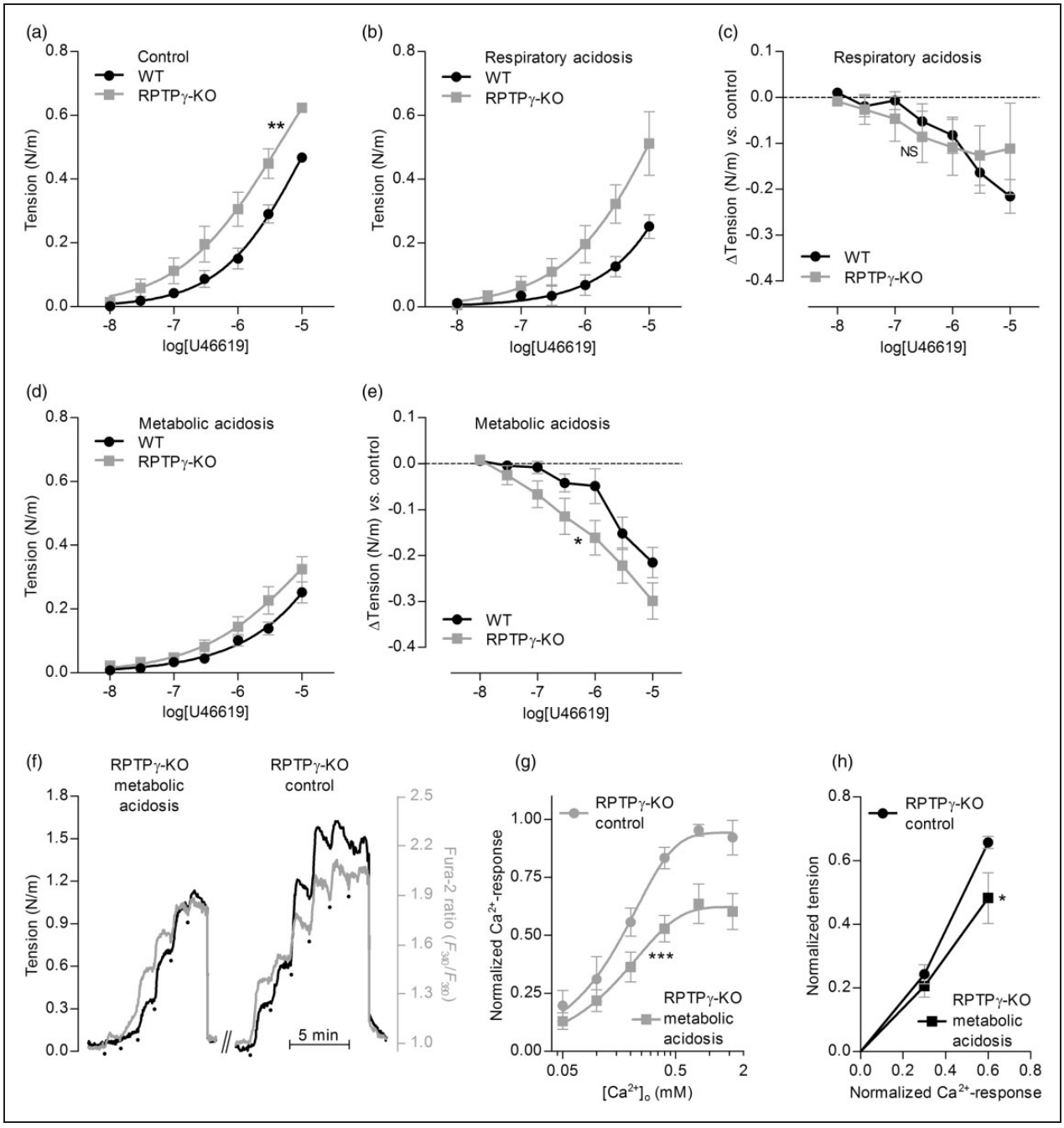

Figure 6. Under equilibrated conditions, RPTPγ is required for HCO_{3o}^- sensing—such that basilar arteries from RPTPγ-knockout mice are hypercontractile—and vasorelaxation in response to metabolic acidosis is explained by a combination of attenuated Ca^{2+} uptake and reduced Ca^{2+}-sensitivity. (a–e) Comparison of U46619-induced contractile responses of basilar arteries from RPTPγ-knockout (KO, n=8) *vs.* wild-type (WT, $n = 10$) mice. Panel (a) summarizes experiments under control conditions (pH_o=7.4, $[HCO_3^-]_o$=22 mM, CO_2=5%); panel (b), under respiratory acidosis (pH_o=7.1, $[HCO_3^-]_o$=22 mM, CO_2=10%); and panel (d), under metabolic acidosis (pH_o=7.1, $[HCO_3^-]_o$=11 mM, CO_2=5%). Panels (c) and (e) summarize the average differences in U46619-induced contractile responses in basilar arteries from RPTPγ-knockout and wild-type mice during respiratory (panel c) and metabolic (panel e) acidosis compared to control conditions. (f) Representative time courses of arterial tension development and VSMC Ca^{2+}-dependent fluorescence signal in Ca^{2+}-depleted basilar arteries from RPTPγ-knockout mice in response to step-increases of $[Ca^{2+}]_o$ during constant exposure to 10 μM U46619. We perform experiments under control conditions (pH_o=7.4, $[HCO_3^-]_o$=22 mM, CO_2=5%) and during metabolic acidosis (pH_o=7.1, $[HCO_3^-]_o$=11 mM, CO_2=5%). The dots indicate step-wise increments in $[Ca^{2+}]_o$ from 0 to 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 mM. (g) Average Ca_i^{2+}-responses from experiments ($n = 5$) like that in panel (f). (h) Average tension development of basilar arteries from RPTPγ-knockout mice ($n = 5$) as a function of the VSMC Ca_i^{2+}-response under control

(Continued)

anti-contractile effect of changing from physiological control conditions (i.e. pH_o 7.4, 22 mM HCO_{3o}^-, 5% CO_2) to an equilibrated acidosis, we observe that the vasorelaxant response to respiratory acidosis (i.e. pH_o 7.1, 22 mM HCO_{3o}^-, 10% CO_2) is equivalent in basilar arteries from wild-type and RPTPγ-knockout mice (Figure 6c). In contrast, vasorelaxation in response to metabolic acidosis (i.e. pH_o 7.1, 11 mM HCO_{3o}^-, 5% CO_2) is potentiated in basilar arteries from RPTPγ-knockout compared to wild-type mice (Figure 6e). These findings support that RPTPγ limits vasorelaxation in response to metabolic acidosis.

Effect of metabolic acidosis on VSMC Ca^{2+}-sensitivity in basilar arteries

The OOE data presented above (Figures 2, 3 and 5) support the conclusion that—in addition to direct effects of H_o^+ and HCO_{3o}^- on cerebral artery tone—indirect effects caused by changes in pH_i also play a role for vasomotor control of basilar arteries. It has been debated to what extent changes in pH_i contribute to vasorelaxation in response to extracellular acid–base disturbances,[27,28] but previous studies have not been able to take into account potential direct effects of changes in $[HCO_3^-]_o$. During metabolic acidosis (i.e. pH_o 7.1, 11 mM HCO_{3o}^-, 5% CO_2), VSMC pH_i decreases by 0.16 ± 0.03 at rest and 0.18 ± 0.03 during maximal contraction (with 10 μM U46619) compared to control conditions ($n = 5$). This degree of intracellular acidification is expected to inhibit vasoconstriction by lowering VSMC Ca^{2+}-sensitivity.[5,12,18,26] To explore mechanisms of vasomotor control additional to RPTPγ-dependent signaling, we next investigate whether metabolic acidosis interferes with VSMC Ca^{2+}-sensitivity in basilar arteries from RPTPγ-knockout mice (Figure 6(f) and (h)). Because U46619 is expected to increase VSMC Ca^{2+}-sensitivity,[29] we determine the contractile response to varying levels of $[Ca^{2+}]_i$ during constant exposure to a maximal stimulatory level of U46619 (10 μM). Our approach is to first deplete the arteries of Ca_i^{2+} and then to expose them to stepwise increases in $[Ca^{2+}]_o$ (Figure 6f). Metabolic acidosis attenuates the VSMC Ca^{2+} response (Figure 6(f) and (g)), but the inhibitory effect of metabolic acidosis on active tension development is greater than what one would expect from the lower $[Ca^{2+}]_i$ levels alone (Figure 6(f) and (h)). Based on these findings, we conclude that—in addition to direct effects of H_o^+ and HCO_{3o}^-—extracellular acidosis also affects tone development by lowering VSMC Ca^{2+}-sensitivity, and this mechanism is likely caused by the concomitant decrease in pH_i.

Discussion

Metabolic regulation of cerebral artery tone is important in the adaptation of cerebral blood flow to local metabolic demand. Although dilation of systemic resistance arteries in response to extracellular acidosis was first described more than a century ago,[2] the complex nature of acid–base disturbances—with concomitant changes in pH_o, $[HCO_3^-]_o$, pCO_2 and other buffer systems—has previously prevented a comprehensive mechanistic understanding.

In the present study, we circumvent previous experimental limitations by utilizing OOE CO_2/HCO_3^- solutions and thereby determining vascular effects of H_o^+, HCO_{3o}^-, and CO_2 one at a time, while holding the other two parameters fixed. We show for the first time that HCO_{3o}^- directly modifies arterial tone, and that H_o^+ causes vasorelaxation even with normal pCO_2 and $[HCO_3^-]_o$. Although, under the conditions of the assay, we find no evidence for a direct effect of CO_2, we corroborate that secondary changes in pH_i induced by extracellular acid-base disturbances modulate arterial tone development. The novel vasomotor response to changes in $[HCO_3^-]_o$ requires a sensing mechanism involving RPTPγ, which—based on its homology to carbonic anhydrases—likely binds HCO_3^- and initiates intracellular signaling in response to acid-base disturbances. To investigate physiological and potential pathophysiological roles of RPTPγ, we investigate vasomotor effects of RPTPγ under equilibrated conditions and demonstrate a HCO_{3o}^--sensitive net braking action of RPTPγ on the vasorelaxant response to metabolic acidosis. The important new insights from the current study and the proposed signaling mechanisms controlling metabolic regulation of artery tone are summarized in Figure 7.

Extracellular HCO_3^- and CO_2

In physiological solutions, CO_2 and HCO_3^- are key buffers and, in addition to H^+, they can modify the vascular response to acid–base disturbances. Our findings

Figure 6. Continue.
conditions (pH_o=7.4, $[HCO_3^-]_o$=22 mM, CO_2=5%) and during metabolic acidosis (pH_o=7.1, $[HCO_3^-]_o$=11 mM, CO_2=5%). The curves in panels (a), (b), (d) and (g) are the results of least-squares fits to sigmoidal functions; in panels (a) and (g), we compare them using extra sum-of-squares *F*-tests. The data points in panels (c), (e) and (h) are compared by repeated-measures two-way ANOVA; in panel (h), we report the significance level based on Bonferroni post-tests. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NS: not significantly different *vs.* wild-type (panels (a), (c) and (e)) or control conditions (panels (g) and (h)).

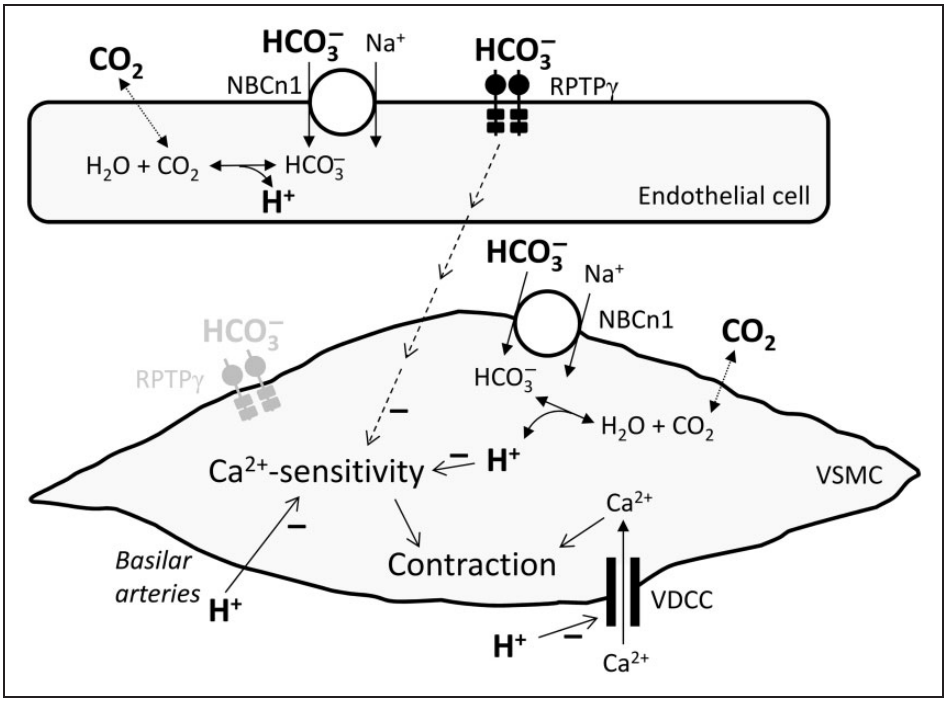

Figure 7. Schematic of proposed signaling pathways affecting cerebral vascular tone in response to acid-base disturbances. Note that in the cases of endothelium-mediated RPTPγ-dependent actions of HCO_{3o}^- and the effect of H_o^+ acting on Ca^{2+}-sensitivity (which could occur via a parallel change in pH_i), we have only seen effects with reducing the concentrations. RPTPγ in the vascular smooth muscle cell is shown in gray because we have evidence for expression but no function has yet been demonstrated. VDCC, voltage-dependent Ca^{2+}-channels; VSMC, vascular smooth muscle cell.

show that lowering $[HCO_3^-]_o$ directly increases vascular tone (Figures 2(d) and 5(f) and Supplemental Figure 1(d)) via a mechanism that requires RPTPγ (Figure 5(b) and (g)) and elevates VSMC Ca^{2+} sensitivity (Figure 2(d) and (g) and Supplemental Figure 1(d) and (g)). The regulation of vasomotor tone by $[HCO_3^-]_o$ is endothelium-dependent: under control conditions, omission of CO_2/HCO_3^- potentiates vasoconstriction to U46619 in basilar arteries from wild-type (Figure 5f) but not RPTPγ-knockout (Figure 5g) mice; however, following inhibition of well-accepted pathways for endothelium-dependent vasorelaxation (i.e. NO, PGI_2 and EDH), omission of CO_2/HCO_3^- no longer affects tension development in basilar arteries from either wild-type or RPTPγ-knockout mice (Figure 5(h) and (i)). The endothelium-dependent regulation of VSMC Ca^{2+}-sensitivity by HCO_{3o}^- is consistent with previously demonstrated inhibitory effects of the endothelium—and endothelium-derived relaxant factors—on cell signaling pathways for VSMC Ca^{2+}-sensitization.[30–33] High transcriptional activity for *Ptprg* in ECs of basilar compared to mesenteric arteries (Figure 4 *vs.* Supplemental Figure 2) is reflected in the magnitude of the vasomotor response to lowering $[HCO_3^-]_o$, which is prominent and RPTPγ-dependent in basilar arteries (Figures 2, 5 and 6) but much less pronounced in mesenteric arteries (Supplemental Figure 1). Selective decreases in $[HCO_3^-]_o$—induced with OOE solutions at a fixed pH_o of 7.4 and CO_2 of 5%—do not give rise to statistically significant changes in VSMC pH_i or $[HCO_3^-]_i$ (Figure 2(a) and (b) and Supplemental Figure 1(a) and (b)), supporting that the novel pro-contractile effect of lowering $[HCO_3^-]_o$ does not require a change in cellular uptake (Figure 7). Extracellular action of HCO_3^- is also supported by the observation that the HCO_{3o}^--dependent vasocontractile effect relies completely on RPTPγ (Figure 5b), which has a carbonic anhydrase-like domain with known extracellular orientation.[15]

The OOE effect of lowering $[HCO_3^-]_o$ is paradoxical because of the well-known vasorelaxation caused by lowering $[HCO_3^-]_o$ under equilibrated conditions (i.e. metabolic acidosis). In cerebral arteries, this paradoxical action: (a) limits vasorelaxation during metabolic acidosis (Figure 6e) and hence may protect against cerebral edema, and (b) could maintain sufficient Ca^{2+}-sensitivity to permit VSMC responses to Ca^{2+}-dependent modulators even under acidotic conditions. Note that this novel HCO_{3o}^--sensitive, RPTPγ-dependent signaling pathway appears to be fully activated

at the control $[HCO_3^-]_o$ of 22 mM. Thus, further increases in $[HCO_3^-]_o$ do not affect tone (Figure 2(d) and Supplemental Figure 1(d)).

In addition to its effects as an extracellular-signaling molecule, HCO_{3o}^- also is important as substrate for the electroneutral Na^+, HCO_3^--cotransporter NBCn1 (Slc4a7), which maintains a relatively alkaline pH_i.[34] Lowering $[HCO_3^-]_o$ under OOE conditions at pH_o 7.1 has no net effect on tone (Figure 3c) because of the competing (a) RPTPγ-dependent tendency toward vasoconstriction and (b) fall in pH_i that inhibits VSMC Ca^{2+}-sensitivity[5,12,18,26] and thus tends to cause vasorelaxation. Accordingly, with increased metabolic activity or decreased perfusion, the integrated effect of the resulting metabolic acidosis would be dilation of feed arteries because the low local pH_o *per se* causes vasorelaxation, whereas the accompanying low $[HCO_3^-]_o$ has no net vasomotor effect. In contrast, lowering $[HCO_3^-]_o$ under OOE conditions at pH_o 7.4 causes vasoconstriction (Figure 2d), and during a partially compensated whole-body metabolic acidosis (e.g. due to renal or metabolic dysfunction), the low $[HCO_3^-]_o$ would tend to limit the degree of acid-induced cerebral vasodilation and thus allow control of cerebral blood flow by normal autoregulatory mechanisms.

Our results show that selective changes in pCO_2 at pH_o 7.4 have no net effect on vasoreactivity (Figure 2e). Clearly, CO_2 has indirect effects on artery tone by changing pH_i, and by changing pH_o under equilibrated conditions.

Direct actions of extracellular pH

In both mesenteric and basilar arteries, a selective decrease in pH_o inhibits the VSMC Ca^{2+}-response and tone development in response to depolarization (Figure 2(h) and Supplemental Figure 1(h)) and agonist-stimulation (Figure 2(c) and Supplemental Figure 1(c)). These results are consistent with previous findings showing that acidosis inhibits voltage-dependent Ca^{2+} channels.[35] However, while a selective increase in pH_o enhances U46619-induced contractions in basilar arteries (Figure 2c), this alkalosis has no effect on norepinephrine-induced contractions in mesenteric arteries (Supplemental Figure 1c) or on depolarization-induced contractions in either vascular bed (Figure 2(h) and Supplemental Figure 1(h)). Because the alkalosis-induced augmentation of agonist-induced contractions of basilar arteries does not involve a change in $[Ca^{2+}]_i$ (Figure 2f), our findings suggest that extracellular alkalosis raises VSMC Ca^{2+}-sensitivity in basilar but not mesenteric arteries. The link between increased pH_o and increased Ca^{2+}-sensitivity remains unclear, but does not involve RPTPγ, as evidenced by the observation that both increases and decreases in pH_o continue to evoke their usual tension responses in basilar arteries from RPTPγ-knockout mice (Figure 5a). It is possible that the effect of high pH_o on Ca^{2+}-sensitivity is secondary to the concomitant intracellular alkalinization (Figure 2a).

The role of intracellular pH in the response to extracellular acid-base disturbances

In addition to direct effects of H_o^+ and HCO_{3o}^- on vascular tone, our data suggest that extracellular acid–base disturbances also act via secondary changes in VSMC pH_i (Figure 7). Investigators have argued both for[27] and against[28] a major role for VSMC pH_i-changes in vascular responses to extracellular acid–base disturbances. Our present study—employing arteries from RPTPγ-knockout mice to eliminate direct effects of HCO_{3o}^-—supports the conclusion that part of the vasorelaxant response to metabolic acidosis occurs via changes in VSMC pH_i: we find that during extracellular acidification, the pH_i of VSMCs is sensitive to changes in $[HCO_3^-]_o$ and pCO_2 (Figure 3a) and that VSMC intracellular acidification is accompanied by decreased VSMC Ca^{2+}-sensitivity (Figure 2 *vs.* Figures 3, 5 and 6). The ability of a decrease in $[HCO_3^-]_o$ to decrease VSMC pH_i and VSMC Ca^{2+}-sensitivity at low pH_o (Figures 3 and 6) is consistent with previous observations that reduced net HCO_3^- uptake consequent to CO_2/HCO_3^- omission,[5,12,26] siRNA-mediated knockdown[5] or knockout[12,18] of NBCn1 substantially lowers VSMC pH_i and inhibits VSMC Ca^{2+}-sensitivity in murine arteries. During vasoconstriction, NBCn1 in VSMCs is stimulated via Ca^{2+}-dependent activation of calcineurin, which protects against intracellular acidification[36] and thereby permits the increased VSMC Ca^{2+}-sensitivity observed during prolonged contraction.[5]

Conclusion

Our use of OOE CO_2/HCO_3^- solutions and RPTPγ-knockout mice allows us to show for the first time that decreases in $[HCO_3^-]_o$ have direct, RPTPγ-dependent vasocontractile effects mediated by increases in VSMC Ca^{2+}-sensitivity. Changes in pH_o also modulate vasomotor responses, partly through acid-induced inhibition of voltage-dependent Ca^{2+}-channels. Finally, the combination of low pH_o and high pCO_2 (respiratory acidosis) or low pH_o and low $[HCO_3^-]_o$ (metabolic acidosis) cause pH_i decreases that lower VSMC Ca^{2+}-sensitivity and thus inhibit vasoconstriction. We propose that these responses to acid–base disturbances collectively fine-tune vascular tone and adjust blood flow to the local metabolic demand.

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Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contributions

EB, CA and WFB conceived the project. EB, DMBB and WFB designed experiments. EB and KBH performed experiments. EB analyzed the data. EB, DMBB, CA and WFB interpreted data. EB drafted the manuscript. All authors revised the manuscript for important intellectual content and approved the final version.

Supplementary material

Supplementary material for this paper can be found at http://jcbfm.sagepub.com/content/by/supplemental-data.

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