Evidence from renal proximal tubules that \( \text{HCO}_3^- \) and solute reabsorption are acutely regulated not by pH but by basolateral \( \text{HCO}_3^- \) and \( \text{CO}_2 \)

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Respiratory acidosis, a decrease in blood pH caused by a rise in \( \text{CO}_2 \), rapidly triggers a compensatory response in which the kidney markedly increases its secretion of \( \text{H}^+ \) from blood to urine. However, in this and other acid-base disturbances, the equilibrium \( \text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HCO}_3^- + \text{H}^+ \) makes it impossible to determine whether the critical parameter is \( \text{CO}_2 \), \( \text{HCO}_3^- \), and/or pH. Here, we used out-of-equilibrium \( \text{CO}_2/\text{HCO}_3^- \) solutions to alter basolateral (BL) \( \text{HCO}_3^- \), \( \text{CO}_2 \), or pH, systematically and one at a time, on isolated perfused S2 rabbit proximal tubules. We found that increasing \( [\text{HCO}_3^-]_\text{BL} \) from 0 to 44 mM, at a fixed \( [\text{CO}_2]_\text{BL} \) of 5% and a fixed \( \text{pH} \), caused \( \text{HCO}_3^- \) reabsorption \( (J_{\text{HCO}_3^-}) \) to fall by half but did not significantly affect volume reabsorption \( (J_v) \). Increasing \( [\text{CO}_2]_\text{BL} \) from 0% to 20%, at a fixed \( [\text{HCO}_3^-]_\text{BL} \) of 22 mM and \( \text{pH} \), caused \( J_{\text{HCO}_3^-} \) to rise 2.5-fold but did not significantly affect \( J_v \). Finally, increasing \( \text{pH} \) from 6.80 to 8.00, at a fixed \( [\text{HCO}_3^-]_\text{BL} \) of 22 mM and \( [\text{CO}_2]_\text{BL} \) of 5%, did not affect either \( J_{\text{HCO}_3^-} \) or \( J_v \). Analysis of the \( J_{\text{HCO}_3^-} \) and \( J_v \) data implies that, as the tubule alters \( J_{\text{HCO}_3^-} \), it compensates the reabsorption of other solutes to keep \( J_v \) approximately constant. Because the cells cannot respond acutely to pH changes, we propose that the responses of \( J_{\text{HCO}_3^-} \) and the reabsorption of other solutes to changes in \( [\text{HCO}_3^-]_\text{BL} \) or \( [\text{CO}_2]_\text{BL} \) involve sensors for basolateral \( \text{HCO}_3^- \) and \( \text{CO}_2 \).

Materials and Methods

Except for the compositions of most of the OOE \( \text{CO}_2/\text{HCO}_3^- \) solutions, our methods are described in detail in ref. 17.

Tubule Perfusion. We hand dissected kidneys from “pathogen-free” female New Zealand White rabbits (1.4–2.0 kg) to yield individual segments of midcortical S2 PTs in accordance with an approved animal protocol. We used two assemblies of concentric glass pipettes to perfuse lumens of single isolated PTs at 37°C, as described by Burg and coworkers (18) and modified by Quigley and Baum (19). We used a calibrated collection pipette (volume = 55 nl) to collect samples of fluid that had flowed down the PT lumen; the luminal collection rate was 12.6 ± 0.3 nl/min \((n = 50)\) (measurement). We superfused the basolateral surface of the PT at 7 ml/min.

Solutions. Table 1 lists the compositions of the solutions. We dissected PTs in Hanks’ solution (solution 1) at 4°C (20). During tubule perfusion, the luminal perfusate always was solution 2, which contained \(^{3}\text{H}\)methoxyinulin (molecular mass = 7,146 Da; catalog no. NET-086L, PerkinElmer). We diazyl \(^{3}\text{H}\)methoxyinulin for 72 h by using a membrane with a molecular mass cutoff of 3,500 Da. After establishing luminal perfusion, we allowed a 20- to 30-min warmup period in which solution 3 flowed through the bath (i.e., basolateral solution) at 37°C. We then changed the bath to either solution 4 (Fig. 1L) or to solution 7 (Fig. 1B and C), which differed only in \([\text{Cl}^-]\), achieved by replacing NaCl with Na gluconate. In control experiments (data not shown), we found that \( J_v \) and \( J_{\text{HCO}_3^-} \) were indistinguishable.

Abbreviations: BL, basolateral; \( J_{\text{HCO}_3^-} \), rate of \( \text{HCO}_3^- \) reabsorption; \( J_v \), rate of volume reabsorption; \( J_{\text{HCO}_3^-}/J_v \rightleftharpoons \text{HCO}_3^- \) and \( \text{CO}_2 \) as potential signals. Using the method that our laboratory developed for generating out-of-equilibrium (OOE) \( \text{CO}_2/\text{HCO}_3^- \) solutions (16), we can now approach the problem by independently varying basolateral \([\text{HCO}_3^-] \), \([\text{CO}_2] \), and \( \text{pH} \).

In the present study, we perfused single, isolated S2 segments of rabbit PTs and collected the fluid that had passed along the PT lumen. Analysis of this collected fluid allowed us to compute volume reabsorption \( (J_v) \); \( \text{HCO}_3^- \) reabsorption \( (J_{\text{HCO}_3^-}) \), which is virtually the same as the \( \text{H}^+ \)-secretion rate under the conditions of our experiments; and the reabsorption of solutes other than NaHCO\(_3\) \((J_{\text{Other}})\). We made the surprising observation that, at least in the short term, \( J_{\text{HCO}_3^-} \) and \( J_{\text{Other}} \) do not respond to changes in basolateral or intracellular pH. The most straightforward hypothesis is that PT cells have sensors for basolateral \( \text{HCO}_3^- \) and a parameter related to \( \text{CO}_2 \).

A major task of the kidney is to secrete \( \text{H}^+ \) into the urine. Inadequate \( \text{H}^+ \) secretion caused, for example, by mutations to acid-base transporters (1–4) or carbonic anhydrases (5), or by renal failure (6, 7), can lead to a life-threatening decrease in blood pH. Moreover, to maintain a stable blood pH, the kidney must appropriately increase \( \text{H}^+ \) secretion in response to metabolic acidosis (a decrease in blood pH caused by a decrease in \([\text{HCO}_3^-]\) at a fixed \([\text{CO}_2]\) or to respiratory acidosis. However, a half century after the classical observation that respiratory acidosis rapidly stimulates renal \( \text{H}^+ \) secretion (8, 9), we still have little insight into how the kidney senses acute acid-base disturbances.

The renal proximal tubule (PT) reabsorbs (from lumen to blood) a liquid that contains \( \approx 80\% \) of the \( \text{HCO}_3^- \) filtered by the glomerulus. The PT cell does this reabsorption by secreting \( \text{H}^+ \) into the PT lumen and using this \( \text{H}^+ \) to titrate luminal \( \text{HCO}_3^- \) to \( \text{CO}_2 \) and \( \text{H}_2\text{O} \). After entering the cell across the apical membrane, the \( \text{CO}_2 \) and \( \text{H}_2\text{O} \) recombine to produce \( \text{H}^+ \) and \( \text{HCO}_3^- \). The cell extrudes the \( \text{H}^+ \) into the lumen across the apical membrane through \( \text{Na}^-/\text{H}^+ \) exchangers (10–12) and \( \text{H}^+ \) pumps (13) and moves the \( \text{HCO}_3^- \) out across the basolateral membrane via the electrogenic \( \text{Na}^+/\text{HCO}_3^- \) cotransporter NBCe1-A (14, 15). Carbonic anhydrases catalyze the interconversions between \( \text{CO}_2 \) and \( \text{H}_2\text{O} \) on the one hand and \( \text{HCO}_3^- \) and \( \text{H}^+ \) on the other (5).

Because blood pH is the parameter regulated by these acid-base transport processes, blood pH or, more likely, intracellular pH (pHi), has been thought to be the parameter sensed by renal cells. However, because of the interconversion \( \text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HCO}_3^- + \text{H}^+ \), it had been impossible to distinguish pH unambiguously from \([\text{HCO}_3^-]\) and \( \text{CO}_2 \) as potential signals. Using the method that our laboratory developed for generating out-of-equilibrium (OOE) \( \text{CO}_2/\text{HCO}_3^- \) solutions (16), we can now approach the problem by independently varying basolateral \([\text{HCO}_3^-]\), \([\text{CO}_2]\), and \( \text{pH} \).

From the compositions of most of the OOE \( \text{CO}_2/\text{HCO}_3^- \) solutions, our methods are described in detail in ref. 17.

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### Table 1. Physiological solutions

<table>
<thead>
<tr>
<th>Solution</th>
<th>NaCl (mM)</th>
<th>KCl (mM)</th>
<th>CaCl₂ (mM)</th>
<th>MgSO₄ (mM)</th>
<th>Glucose (mM)</th>
<th>NaHCO₃ (mM)</th>
<th>Hepes (mM)</th>
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<td>0</td>
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</tr>
<tr>
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<td>0.8</td>
<td>0</td>
<td>0</td>
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<td>7.40</td>
</tr>
<tr>
<td>5</td>
<td>137.7</td>
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<td>0</td>
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<td>7.40</td>
</tr>
<tr>
<td>6</td>
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<td>5</td>
<td>0.2</td>
<td>0.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7.40</td>
</tr>
<tr>
<td>7</td>
<td>113</td>
<td>5</td>
<td>0.2</td>
<td>0.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>10</td>
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<tr>
<td>11</td>
<td>113</td>
<td>10</td>
<td>0.2</td>
<td>0.8</td>
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<tr>
<td>12</td>
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<td>10</td>
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<tr>
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<td>0.8</td>
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<td>0</td>
<td>0</td>
<td>7.40</td>
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</tbody>
</table>

The concentrations are in mM except for CO₂ (given both in mM and %) and albumin (g/liter). Except for solution 1 (4-CI), all solutions were titrated to the indicated pH at 37°C. The HCl and HEPES were titrated with NaOH.

We generated OOE CO₂/HCO₃⁻ solutions by rapidly mixing streams of two dissimilar solutions (17), delivering the newly mixed solution to the PT within ~200 ms. Fig. 1C in ref. 17 shows the detailed compositions of two newly mixed OOE solutions and the minute degree of equilibration that occurs during the ~200-ms interval. The final compositions of OOE solutions in Fig. 1A (solutions 5 and 6) were the same as for solution 4 except that we replaced HCO₃⁻ with gluconate or vice versa to keep [Cl⁻] constant. The final compositions of the OOE solutions in Fig. 1B (solutions 7–11) were the same as for solution 7 except for [CO₂]. The final compositions of the OOE solutions in Fig. 1C (solutions 12 and 13) were the same as for solution 7 except for pH, the concentrations of neutral and anionic HEPES, and the [NaCl] (which we adjusted to keep osmolality constant).

We used solution 14 only in experiments in which we measured pH. Here, solution 14 was present in the lumen before we switched to solution 2. In addition, solution 14 was present in the bath when a CO₂/HCO₃⁻-containing solution (i.e., solutions 4–13) was not present.

### Measurement of Jᵥ and JHCO₃⁻

We measured JHCO₃⁻ (pmol/min per mm tubule length) and Jᵥ (nl/min⁻¹·mm⁻¹) by using an approach similar to that of McKinney and Burg (21). We assayed total CO₂ in aliquots of the perfusate and collected fluid by using a NanoFlo microfluorometer (World Precision Instruments, Sarasota, FL) and reagents (Diagnostic Kit 132-A) from Sigma-Aldrich (22, 23). Luminal [³H]methoxyinulin served as a volume marker for calculating Jᵥ.

Each experiment consisted of two data collection periods. The bath contained equilibrated CO₂/HCO₃⁻ (solutions 4 or 7) during the first period and an OOE solution (solutions 5, 6, 8, 9, 10, 11, 12, or 13) during the second. In control experiments in which identical equilibrated CO₂/HCO₃⁻ solutions (solution 7) were present during both periods, JHCO₃⁻ values were identical, as were Jᵥ values (17). In each experiment, we divided the JHCO₃⁻ (and Jᵥ) value obtained during the second (OOE) collection period by the comparable values obtained during the first (control) period to generate OOE/control ratios for JHCO₃⁻ (and Jᵥ). In Fig. 1, JHCO₃⁻ (and Jᵥ) values for control conditions (triangles) are raw mean values; each OOE value (circles, squares, and diamonds) is the product of the raw mean JHCO₃⁻ (or Jᵥ) value and the average OOE/control ratio for JHCO₃⁻ (or Jᵥ).

### Calculation of [HCO₃⁻] in the Fluid Reabsorbed by the PT

In experiments in which we varied [HCO₃⁻] in solution 4 (solutions 8 and 11), the bath was used as a baseline perfusate. We used this baseline to generate OOE/control ratios of JHCO₃⁻ (and Jᵥ). In solution 4, JHCO₃⁻ (and Jᵥ) was used as a baseline perfusate. Solution 4 was replaced with NaCl in solution 6.140, Jᵥ was 7.40 for solutions 4, 6, 11, and 14, and JHCO₃⁻ was 0.40 for solution 14.

To calculate [HCO₃⁻] in the reabsorbed fluid, we used the equation to compute [HCO₃⁻] in the reabsorbed fluid as the ratio JHCO₃⁻/Jᵥ (Fig. 2 Lower).

\[
\frac{2 \times J_{HCO_3^-} + J_{Other}}{J_v} = 300 \text{ mosM}.
\]

Here, \(2 \times J_{HCO_3^-}\) is the reabsorption of NaHCO₃, and \(J_{Other}\) is the reabsorption of solutes other than NaHCO₃. We used this equation to compute \(J_{Other}\) for each value of [HCO₃⁻] in solution 4 and [CO₂] in solution 2. Finally, knowing \(J_{Other}\) and \(J_v\), we computed the concentration of all other solutes in the reabsorbed fluid as the ratio \(J_{Other}/J_v\) (Fig. 2 Lower).
Measurement of \( \text{pH}_i \). We calculated \( \text{pH}_i \) from the fluorescence excitation ratio of \( 2',7'\text{-bis-(2-carboxyethyl)-5(and-6)carboxy-fluorescein} \) (24), loaded into cells as the acetoxymethyl ester (no. B-1170, Molecular Probes) (17). The inverted microscope was a Zeiss IM-35, equipped with apparatus for epi-illumination, a 40\( \times \)/NA 0.85 objective, dual filter wheels (Ludl Electronic Products, Hawthorne, NY) for alternating between 495 ± 5 nm and 440 ± 5 nm excitation filters (Thermo Oriel, Stratford, CT), a 510-nm long-pass dichroic mirror, a 530-nm long-pass filter, an image intensifier (KS-1381 intensifier, Videoscope, Dulles, VA), and a camera (CCD 72, Dage–M.T.I., Michigan City, IN). We converted the \( I_{490}/I_{440} \) ratios to \( \text{pH}_i \) values by using the high-K\(^+\)/nigericin technique (25), as modified for one-point calibrations (26).

Supporting Information. For additional information on results and discussion, see Figs. 5 and 6, Table 2, and Supporting Text, which are published as supporting information on the PNAS web site.

Results

Effect of Variations in \([\text{HCO}_3^-]_{\text{BL}}\). In the Fig. 1A Top, the triangle \(( [\text{HCO}_3^-]_{\text{BL}} = 22 \text{ mM}) \) represents \( J_{\text{HCO}_3^-} \), with all three basolateral acid-base parameters at their equilibrated, physiological values. Switching to an OOE basolateral (i.e., bath) solution that was nominally \( \text{HCO}_3^- \)-free, while holding \([\text{CO}_2]_{\text{BL}}\) and \( \text{pH}_{\text{BL}} \) at their physiological values, caused \( J_{\text{HCO}_3^-} \) to increase by \( \approx 50\% \). This maneuver approximates the “metabolic” metabolic acidosis. Conversely, switching from the equilibrated 5% \( \text{CO}_2/22 \text{ mM HCO}_3^- \) at \( \text{pH} 7.40 \) solution to an OOE bath solution with twice the normal \( [\text{HCO}_3^-]_{\text{BL}} \), but with \([\text{CO}_2]_{\text{BL}}\) and \( \text{pH}_{\text{BL}} \) at their physiological values, caused \( J_{\text{HCO}_3^-} \) to fall by \( \approx 30\% \). Thus, changes in \([\text{HCO}_3^-]_{\text{BL}} \) cause \( J_{\text{HCO}_3^-} \) to change in a direction that would help the PT to respond appropriately to stabilize blood pH.

The fluid that the PT reabsorbs is nearly isosmotic, and the calculated \([\text{HCO}_3^-] \) in this reabsorbed fluid is the ratio \( J_{\text{HCO}_3^-}/ \)}
...cause graded decreases in steady-state pHi. Thus, isolated changes in [CO2]BL cause significant changes in Jv. Therefore, JHCO3/Jv (i.e., [HCO3\textsuperscript{-}] in the reabsorbate) must vary considerably. In Fig. 1A, this calculated [HCO3\textsuperscript{-}] was 159 mM at a [HCO3\textsuperscript{-}] of 0 mM (i.e., the reabsorbate was isotonic NaHCO3). In Fig. 2A Upper, we replotted the JHCO\textsubscript{3} data from Fig. 1A Top and also plot the flux of all other solutes, as described in Materials and Methods. Fig. 2A Lower summarizes the computed reabsorbate values of [NaHCO3] and all other solutes. The analysis in Fig. 2A illustrates that increasing [HCO3\textsuperscript{-}] not only lowers JHCO3, but reciprocally raises the reabsorption of other solutes (J\textsubscript{Other}), the appropriate response for maintaining a constant Jv and, thus, a constant blood pressure.

Because extracellular acid-base disturbances generally cause pHi to change (27), we examined how changes in [HCO3\textsuperscript{-}] affect the steady-state pHi of PT cells under the same conditions that prevailed for Fig. 1A Top and Middle. As shown in the Fig. 1A Bottom, an increase in [HCO3\textsuperscript{-}] from 0 to 22 mM caused steady-state pHi to increase by 0.32. However, further increasing [HCO3\textsuperscript{-}] to 44 mM did not cause a statistically significant increase in steady-state pHi.

**Effect of Isolated Variations in [CO2]BL.** In Fig. 1B Top, the triangle ([CO2]BL = 5%) represents virtually the same conditions as the triangle in Fig. 1A Top. Switching from this equilibrated, physiological solution to one in which we increased [CO2]BL to four times its physiological value, while holding [HCO3\textsuperscript{-}]\textsubscript{BL} and pHi\textsubscript{BL} at their physiological values, caused JHCO3 to rise by ~50%. This maneuver approximates the "respiratory" half of respiratory acidosis. If we instead exposed the PT to a nominally CO2-free OOE bath solution, while holding [HCO3\textsuperscript{-}]\textsubscript{BL} and pHi\textsubscript{BL} at their physiological values, JHCO3 fell by 40% compared with normal. The small JHCO3 observed in the nominal absence of basolateral CO2 may be due, in part, to CO2 from the lumen reaching the basolateral membrane. The midpoint of the JHCO3 response to CO2 is at a [CO2]BL of ~6%, which is somewhat above the physiological [CO2] of arterial blood and somewhat below that of the renal cortex (28, 29). Thus, isolated changes in [CO2]BL cause JHCO3 to change in a direction that would help the PT to respond appropriately to stabilize blood pH.

Fig. 1B Middle shows that isolated changes in [CO2]BL tended to cause Jv to increase. However, none of the differences between the Jv values obtained in OOE solutions and the Jv value obtained in the equilibrated solution (solution 7) were statistically significant. As [CO2]BL rose from 0% to 20%, the calculated reabsorbate [NaHCO3] rose from 70 mM (i.e., the isosmotic reabsorbate was ~50% NaHCO3) to 129 mM (i.e., the reabsorbate was mainly isotonic NaHCO3). As summarized in Fig. 2B, increases in [CO2]BL not only raise JHCO3 and reabsorbate [NaHCO3] but also reciprocally lower J\textsubscript{Other} and the total concentration of other solutes in the reabsorbate.

We also measured pHi of the PT cells under the conditions of Fig. 1B Top and Middle. Fig. 1B Bottom shows that increases in [CO2]BL cause decreased changes in steady-state pHi, and also that the data in Fig. 1B are consistent with the hypothesis that elevations in [CO2]BL increase JHCO3, indirectly by lowering pHi. According to this hypothesis, the increased JHCO3 that we observed when decreasing [HCO3\textsuperscript{-}]\textsubscript{BL} (Fig. 1A Top) also would have been caused by the attendant decrease in pHi (Fig. 1A Bottom).

**Effect of Variations in pH\textsubscript{BL}.** If pHi changes determine JHCO3, we should be able to increase JHCO3 by lowering pHi, even without changing [HCO3\textsuperscript{-}]\textsubscript{BL} or [CO2]BL. In Fig. 1C Top, the triangle (pHi\textsubscript{BL} = 7.40) represents the same conditions as the triangle in Fig. 1B Top. Surprisingly, switching to either a pH-6.80 or pH-8.00 OOE bath solution, while holding [HCO3\textsuperscript{-}]\textsubscript{BL} and [CO2]BL at their physiological values, caused no change in either JHCO3 (Top) or Jv (Middle). As pHi\textsubscript{BL} rose from 6.80 to 8.00, the calculated [HCO3\textsuperscript{-}]\textsubscript{BL} in the reabsorbate ranged between 96 mM and 99 mM. On the other hand, Fig. 1C Middle shows that raising pHi\textsubscript{BL} from 6.80 to 8.00 caused substantial, graded increases in steady-state pHi. In fact, the ΔpHi/ΔpHi\textsuperscript{BL} ratio for these PT cells (~60%) is among the highest recorded for any cell (30–33).

**Discussion**

**What Contributes to J\textsubscript{Other}?** One of our most striking observations is that JHCO3, and J\textsubscript{Other} (i.e., reabsorption rate of solutes other than HCO3 and its obligated Na\textsuperscript{+}) change reciprocally to keep Jv relatively constant. The Other solutes include (i) Cl\textsuperscript{-}, (ii) organic solutes, and (iii) Na\textsuperscript{+} in excess of that accomplishing HCO3\textsuperscript{-} uptake. Diffusion and solvent drag through tight junctions, possibly augmented by apical Cl-base exchange (34), could contribute to Cl\textsuperscript{-} reabsorption (Fig. 3). Apical cotransport of Na\textsuperscript{+} with glucose, lactate, and glutamine could contribute to the reabsorption of organic solutes. The near constancy of Jv implies that JNa and, thus, the Na-K pump rate, must also be relatively stable.

The PT could produce the highest J\textsubscript{Other} observed in the present study (~143 pmol·min\textsuperscript{-1}·mm\textsuperscript{-2} in Fig. 2A) by reabsorbing ~4.25 mM or ~30% of the 14.5 mM of organic solutes initially present in the lumen (i.e., 10.5 mM glucose, 2 mM lactate, and 2 mM glutamine), along with the coupled Na\textsuperscript{+} and...
Further records of the J, contributes to cell types (37–40). The squares are replots of data from Fig. 1A Top vs. the corresponding pH data from Fig. 1A Bottom. Similarly, the squares are replots of the JHCO3, vs. the pH data from Fig. 1B. For both data sets, increases in pH, whether caused by a rising [HCO3]i, or a falling [CO2]BL, are associated with decreases in JHCO3. However, the replot of the JHCO3, vs. pH data from Fig. 1C, plotted as diamonds in Fig. 4, shows that even large pH changes are not sufficient to alter JHCO3, provided both [HCO3]iBL and [CO2]iBL are constant. Supporting Text shows that large pH changes are likewise insufficient to alter JOther (Fig. 5). Thus, pHi cannot be the parameter triggering reciprocal changes in JHCO3, and JOther in Fig. 2A and B.

In retrospect, the insensitivity of JHCO3, to acute pH changes could have been anticipated for both theoretical and experimental reasons. First, theory predicts that a fall in pH could trigger an increase in JHCO3, only by stimulating both H+ extrusion across the apical membrane and HCO3 exit across the basolateral membrane. However, a fall in pH ought to inhibit NBCe1-A (Fig. 3), either directly or indirectly by lowering [HCO3]i, and [CO2]i, just as a fall in pH inhibits Cl-HCO3 exchange in other cell types (37–40).

Second, previous experiments showed that, with CO2/HCO3 absent from both lumen and bath or present only in the lumen, pH recovers (i.e., increases) slowly from acute intracellular acid loads (41). Just as important, the pH recovery rates are only modestly pH sensitive. On the other hand, with CO2/HCO3 present both in lumen and bath or only in the bath, pH recovers far more rapidly but still with only modest pH sensitivity (see fig. 4 in ref. 41). In other experiments (42), in which CO2/HCO3 was either absent from both lumen and bath (bilateral Heps) or present in both lumen and bath (bilateral CO2/HCO3), a more quantitative analysis of acid-extrusion rates (i.e., the sum of the apical Na-H exchange and H+ pumping) was possible. In bilateral Heps, acid extrusion was low and only modestly pH dependent. In bilateral CO2/HCO3, acid extrusion was again only modestly pH dependent but ~8-fold higher at identical pH values (see fig. 5 in ref. 42). Thus, the main determinant of acid extrusion in intact PTs is not pH, but parameters related to [HCO3]iBL and/or [CO2]iBL.

What, then, is the primary trigger for reciprocal changes in JHCO3, and JOther? A fall in [HCO3]iBL could lower HCO3 backleak through tight junctions (43, 44) and, thereby, raise JHCO3, NBCe1-A (Fig. 3) appears to transport CO3- when operating with a Na+:HCO3 stoichiometry of 1:2 (I. Grichtchenko and W.F.B., unpublished data) and may well transport both CO2 and HCO3 when operating with a 1:3 stoichiometry in the PT. Thus, increases in [HCO3]i and [CO3]i decreases in [HCO3]iBL and [CO2]iBL could stimulate NBCe1-A and thereby raise JHCO3. However, although effects on HCO3 extrusion of NBCe1-A might serve as secondary modulators of JHCO3, it is unclear how these effects could serve as primary triggers for changes in JOther. Let alone closely coordinated, reciprocal changes in JHCO3, and JOther (Fig. 2 Upper).

Even though our data indicate that PT cells do not respond to acute pH changes by modulating JHCO3, or JOther, our data do not address the issue of how prolonged pH changes might affect cells. In opossum-kidney cells, chronic metabolic or respiratory acidosis (pHe, 7.0 vs. 7.4) over a period of 2 days increases Na-H exchange by ~30% (45). This stimulation is accompanied by increased levels of mRNA encoding the Na-H exchanger NHE3 and abrogated either by herbimycin A (inhibitor of certain soluble tyrosine kinases) or by overexpressing csk (46), a physiological inhibitor of c-src.

Do Proximal Tubules Sense Acute pH Changes? The conventional wisdom is that acid-base chemosensitive cells, including central chemoreceptor neurons, peripheral chemoreceptor glomus cells, and certain epithelial cells (e.g., PT cells), sense acute acid-base disturbances through pH changes (36). Although the evidence is consistent with a major role for pH in neurons and glomus cells, our data suggest that this conclusion is not the case in the proximal tubule. The circles in Fig. 4 are replots of the JHCO3, data from Fig. 1A Top vs. the corresponding pH data from Fig. 1A Bottom. Similarly, the squares are replots of the JHCO3, vs. the pH data from Fig. 1B. For both data sets, increases in pH, whether caused by a rising [HCO3]iBL or a falling [CO2]iBL, are associated with decreases in JHCO3. However, the replot of the JHCO3, vs. pH data from Fig. 1C, plotted as diamonds in Fig. 4, shows that even large pH changes are not sufficient to alter JHCO3, provided both [HCO3]iBL and [CO2]iBL are constant. Supporting Text shows that large pH changes are likewise insufficient to alter JOther (Fig. 5). Thus, pH cannot be the parameter triggering reciprocal changes in JHCO3, and JOther in Fig. 2A and B.

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What Does the PT Sense? Supporting Text includes an analysis of how the experimental maneuvers in Fig. 1 influence the intracellular and basolateral values of [CO2], pH, [H2CO3], [HCO3], and [CO3] (Fig. 6). The analysis reveals that no single parameter exhibits a pattern that consistently correlates, positively or negatively, with the patterns of JHCO3, and JOther in Fig. 2 Upper. Moreover, the analysis reveals that the next most parsimonious hypothesis, that the patterns of JHCO3, and JOther in Fig. 2 Upper reflect the sensing of two parameters, is straightforward for only three of 45 parameter pairs: (i) [HCO3]iBL and [CO2]BL (ii) [HCO3]iBL and [CO2], and (iii) [HCO3]iBL and [H2CO3] (Table 2). As summarized in Fig. 3, we propose that PT cells respond to increases in [HCO3]iBL by reducing JHCO3, and raising JOther, and respond to decreases in [CO2]BL (or a related parameter) by raising JHCO3, and reducing JOther.

We already noted that adding CO2/HCO3 to the basolateral, but not the luminal, side of the PT triggers a large increase in H+ extrusion (41, 42). The simplest explanation for these earlier data are that the basolateral CO2 strongly enhanced acid-base transporters, overwhelming the inhibition imposed by the basolateral HCO3. Because adding CO2/HCO3 to the lumen fails to stimulate acid extrusion (41), we propose that CO2 stimulates a sensor at or near the basolateral membrane. In principle, the sensor could face the basolateral fluid and sense [CO2]BL per se, or the sensor could be inside the cell near its basolateral membrane and sense [CO2] or [H2CO3].

In summary, at least during the acute response to acid-base disturbances, the kidney appears to regulate whole-body acid-base balance not by monitoring blood pH per se but by monitoring the levels of the two major buffer components that what...
signals may provide important clues for treating both acid-base disturbances and hypertension.

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