Evidence from renal proximal tubules that HCO_3^- and solute reabsorption are acutely regulated not by pH but by basolateral HCO_3^- and CO_2

Yuehan Zhou, Jinhua Zhao*, Patrice Bouyer, and Walter F. Boron†

Department of Cellular and Molecular Physiology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520

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Respiratory acidosis, a decrease in blood pH caused by a rise in $[CO_2]$, rapidly triggers a compensatory response in which the kidney markedly increases its secretion of H^+ from blood to urine. However, in this and other acid-base disturbances, the equilibrium $CO_2 + H_2O \rightleftarrows HCO_3^- + H^+$ makes it impossible to determine whether the critical parameter is $[CO_2]$, $[HCO_3^-]$, and/or pH. Here, we used out-of-equilibrium CO_2/HCO_3^- solutions to alter basolateral (BL) $[HCO_3^-]$, $[CO_2]$, or pH, systematically and one at a time, on isolated perfused S2 rabbit proximal tubules. We found that increasing $[HCO_3^-]_{BL}$ from 0 to 44 mM, at a fixed $[CO_2]_{BL}$ of 5% and a fixed pH_{BL} of 7.40, caused HCO_3^- reabsorption (J_{HCO_3}) to fall by half but did not significantly affect volume reabsorption (J_V). Increasing $[CO_2]_{BL}$ from 0% to 20%, at a fixed $[HCO_3^-]_{BL}$ of 22 mM and pH_{BL} of 7.40, caused J_{HCO_3} to rise 2.5-fold but did not significantly affect J_V. Finally, increasing pH_{BL} from 6.80 to 8.00, at a fixed $[HCO_3^-]_{BL}$ of 22 mM and $[CO_2]_{BL}$ of 5%, did not affect either J_{HCO_3} or J_V. Analysis of the J_{HCO_3} and J_V data implies that, as the tubule alters J_{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_{HCO_3} and the reabsorption of other solutes to changes in $[HCO_3^-]_{BL}$ or $[CO_2]_{BL}$ involve sensors for basolateral HCO_3^- and CO_2.

kidney | out-of-equilibrium solution | acid-base disturbances | volume reabsorption

A major task of the kidney is to secrete H^+ into the urine. Inadequate renal 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 H^+ secretion in response to metabolic acidosis (a decrease in blood pH caused by a decrease in $[HCO_3^-]$ at a fixed CO_2) or to respiratory acidosis. However, a half century after the classical observation that respiratory acidosis rapidly stimulates renal 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 ≈80% of the HCO_3^- filtered by the glomerulus. The PT cell does this reabsorbtion by secreting H^+ into the PT lumen and using this H^+ to titrate luminal HCO_3^- to CO_2 and H_2O. After entering the cell across the apical membrane, the CO_2 and H_2O recombine to produce H^+ and HCO_3^-. The cell extrudes the H^+ into the lumen across the apical membrane through Na-H exchangers (10–12) and H^+ pumps (13) and moves the HCO_3^- out across the basolateral membrane via the electrogenic Na/HCO_3 cotransporter NBCe1-A (14, 15). Carbonic anhydrases catalyze the interconversions between CO_2 and H_2O on the one hand and HCO_3^- and 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 (pH_i), has been thought to be the parameter sensed by renal cells. However, because of the interconversion $CO_2 + H_2O \rightleftarrows HCO_3^- + H^+$, it had been impossible to distinguish pH unambiguously from $[HCO_3^-]$ and CO_2 as potential signals. Using the method that our laboratory developed for generating out-of-equilibrium (OOE) CO_2/HCO_3^- solutions (16), we can now approach the problem by independently varying basolateral $[HCO_3^-]$, $[CO_2]$, and 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); HCO_3^- reabsorption (J_{HCO_3}), which is virtually the same as the H^+-secretion rate under the conditions of our experiments; and the reabsorption of solutes other than $NaHCO_3$ (J_{Other}). We made the surprising observation that, at least in the short term, J_{HCO_3} and J_{Other} do not respond to changes in basolateral or intracellular pH. The most straightforward hypothesis is that PT cells have sensors for basolateral HCO_3^- and a parameter related to CO_2.

Materials and Methods

Except for the compositions of most of the OOE CO_2/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 J_V/J_{HCO_3} measurements). 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}H]methoxyinulin (molecular mass ≈ 7,146 Da; catalog no. NET-086L, PerkinElmer). We dialyzed the [^{3}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. 1*A*) or to solution 7 (Fig. 1 *B* and *C*), which differed only in $[Cl^-]$, achieved by replacing NaCl with Na gluconate. In control experiments (data not shown), we found that J_V and J_{HCO_3} were indistinguishable

Abbreviations: BL, basolateral; J_{HCO_3}, rate of HCO_3^- reabsorption; J_V, rate of volume reabsorption; J_{Other}, rate of reabsorption of other solutes; OOE, out-of-equilibrium; pH_i, intracellular pH; PT, proximal tubule.

*Present address: Renal Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114-2696

†To whom correspondence should be addressed. E-mail: walter.boron@yale.edu.

Table 1. Physiological solutions

Component	Solution																			
	1	2	3	4	5A	5B	6A	6B	7	8A	8B	9A	9B	10A	10B	11A	11B	12A	12B	13A	13B	14
NaCl	137	124.7	115	79	137.3	20.7	137.3	20.7	101	91	113	113	91	113	91	113	91	54.7	142.1	54.7	132	130.7
KCl	5	5	5	5	0	10	0	10	5	0	10	10	0	10	0	10	0	10	0	10	0	5
Na_2HPO_4	0.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NaH_2PO_4	0	2	2.3	2	4	0	0	0	2	4	0	0	4	0	4	0	4	0	4	0	4	2
$CaCl_2$	0.2	1	3.6	1	2	0	2	0	1	2	0	0	2	2	0	2	0	2	0	0	2	1
$MgSO_4$	0.8	1.2	1	1.2	2.4	0	2.4	0	1.2	2.4	0	0	2.4	0	2.4	0	2.4	0	2.4	0	2.4	1.2
$MgCl_2$	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glucose	0	10.5	8.3	10.5	21	0	21	0	10.5	21	0	0	21	0	21	0	21	0	21	0	21	10.5
Glutamine	2	2	0	2	4	0	4	0	2	4	0	0	4	0	4	0	4	0	4	0	4	2
L-alanine	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L-lactic acid	2	2	2	2	4	0	4	0	2	4	0	0	4	0	4	0	4	0	4	0	4	2
CO_2, mM (%)	0	1.2 (5)	1.2 (5)	1.2 (5)	2.4 (10)	0	0	2.4 (10)	1.2 (5)	0	0	1.2 (5)	0	4.8 (20)	0	9.6 (40)	0	2.4 (10)	0	2.4 (10)	0	0
$NaHCO_3$	0	22	22	22	0	0	0	88	22	0	44	44	0	44	0	44	0	44	0	44	0	0
Na gluconate	0	0	0	22	0	88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tris·HCl	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hepes	0	0	0	32.5	0	65	65	0	32.5	65	0	0	65	0	65	0	65	0	65	0	65	32.5
Albumin, g/liter	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pH	7.40	7.40	7.40	7.40	5.40	7.55	7.29	7.70	7.40	6.99	9.40	7.70	7.34	7.10	7.46	6.80	7.52	7.40	6.64	7.40	8.11	7.40

The concentrations are in mM except for CO_2 (given both in mM and %) and albumin (g/liter). Except for solution 1 (4°C), all solutions were titrated to the indicated pH at 37°C. Tris·HCl and Hepes were titrated with NaOH. Solutions: 1, Hanks' dissection; 2, lumen, equilibriated 5% CO_2, 22 mM HCO_3^-; 3, warmup bath, equilibrated 5% CO_2 22 mM HCO_3^-; 4, 86 mM Cl^- bath, equilibrated 5% CO_2 22 mM HCO_3^-; 5, 86 mM Cl^- bath, OOE solution 5% CO_2 0 mM HCO_3^-; 6, 86 mM Cl^- bath, OOE solution 5% CO_2 44 mM HCO_3^-; 7, standard bath, equilibrated 5% CO_2 22 mM HCO_3^-; 8, bath, OOE solution 0% CO_2 22 mM HCO_3^-; 9, bath, OOE solution 2.5% CO_2 22 mM HCO_3^-; 10, bath, OOE solution 10% CO_2 22 mM HCO_3^-; 11, bath, OOE solution 20% CO_2 22 mM HCO_3^-; 12, pH 6.8 bath, OOE solution 5% CO_2 22 mM HCO_3^-; 13, pH 8.0 bath, OOE solution 5% CO_2 22 mM HCO_3^-; 14, Hepes lumen/bath. Solution 2 was used as a luminal perfusate. Solution 3 was used as a basolateral perfusate. OOE solutions were generated by rapidly mixing their respective A and B components in 1:1 ratio. Solutions 5B, 6A, 8A, 8B, 9B, 10B, 11B, 12B, and 13B were vigorously gassed with 100% O_2 to render them free of CO_2. Mixed pH was 7.40 for solutions 5, 6, 8–11, 6.80 for solution 12, and 8.00 for solution 13.

in solutions 4 and 7. All solutions had osmolalities of 300 ± 2 mosM. Note that solution 7 was identical to luminal solution 2 except for the addition of 32.5 mM Hepes (titrated with NaOH) in solution 7, which replaced an osmotically equivalent amount of NaCl in solution 2.

We generated OOE CO_2/HCO_3^- solutions by rapidly mixing streams of two dissimilar solutions (17), delivering the newly mixed solution to the PT within ≈200 ms. Fig. 1*C* 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. 1*A* (solutions 5 and 6) were the same as for solution 4 except that we replaced HCO_3^- with gluconate or *vice versa* to keep $[Cl^-]$ constant. The final compositions of the OOE solutions in Fig. 1*B* (solutions 7–11) were the same as for solution 7 except for $[CO_2]$. The final compositions of the OOE solutions in Fig. 1*C* (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_i. 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 CO_2/HCO_3^--containing solution (i.e., solutions 4–13) was not present.

Measurement of J_V and J_{HCO_3}. We measured J_{HCO_3} (pmol/min per mm tubule length) and J_V (nl·min^{-1}·mm^{-1}) by using an approach similar to that of McKinney and Burg (21). We assayed total CO_2 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 [^{3}H]methoxyinulin served as a volume marker for calculating J_V.

Each experiment consisted of two data collection periods. The bath contained equilibrated CO_2/HCO_3^- (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 CO_2/HCO_3^- solutions (solution 7) were present during both periods, J_{HCO_3} values were identical, as were J_V values (17). In each experiment, we divided the J_{HCO_3} (and J_V) value obtained during the second (OOE) collection period by the comparable values obtained during the first (control) period to generate OOE/control ratios for J_{HCO_3} (and J_V). In Fig. 1, J_{HCO_3} (and J_V) values for control conditions (triangles) are raw mean values; each OOE value (circles, squares, and diamonds) is the product of the raw mean J_{HCO_3} (or J_V) value and the average OOE/control ratio for J_{HCO_3} (or J_V).

Calculation of $[HCO_3^-]$ in the Fluid Reabsorbed by the PT. In experiments in which we varied $[HCO_3^-]_{BL}$ (Fig. 1*A*) or $[CO_2]_{BL}$ (Fig. 1*B*), we computed J_{HCO_3} (Fig. 1 *A* and *B Top*) and J_V (Fig. 1 *A* and *B Middle*). From these values, we calculated the $[HCO_3^-]$ in the reabsorbate as the ratio J_{HCO_3}/J_V for each $[HCO_3^-]_{BL}$ (Fig. 2*A Lower*) and each $[CO_2]_{BL}$ (Fig. 2*B Lower*). Assuming that the PT reabsorbs fluid isosmotically (300 mosM),

$$\frac{2 \times J_{HCO_3} + J_{Other}}{J_V} = 300 \text{ mosM}. \qquad [1]$$

Here, $2 \times J_{HCO_3}$ is the reabsorption of $NaHCO_3$, and J_{Other} is the reabsorption of solutes other than $NaHCO_3$. We used this equation to compute J_{Other} for each value of $[HCO_3^-]_{BL}$ and $[CO_2]_{BL}$ (Fig. 2 *Upper*). Finally, knowing J_{Other} and J_V, we computed the concentration of all Other solutes in the reabsorbate as the ratio J_{Other}/J_V (Fig. 2 *Lower*).

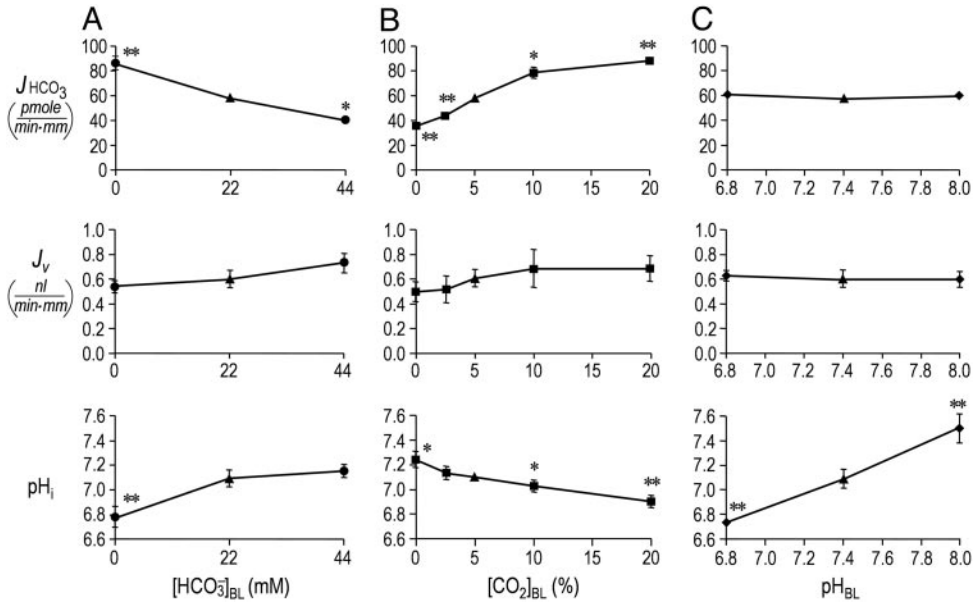

Fig. 1. Effect of isolated changes in basolateral acid-base parameters on J_{HCO_3}, J_V, and pH_i. (*A*) Effect of changing $[HCO_3^-]_{BL}$ at a fixed $[CO_2]_{BL}$ of 5% and a fixed pH_{BL} of 7.40. Data represent mean ± SEM; error bars are omitted when they are smaller than the size of the symbol. For *Top* and *Middle*, in each of the 13 experiments we paired an equilibrated bath CO_2/HCO_3^- solution (▲) with one of two OOE solutions (circles; see *Materials* and *Methods*). $n = 7$, $[HCO_3^-]_{BL} = 0$; $n = 6$, $[HCO_3^-]_{BL} = 44$ mM. For *Bottom*, each pH_i experiment ($n = 6$) included bath exposures to each of the three solutions in *Top*. (*B*) Effect of changing $[CO_2]_{BL}$ at a fixed $[HCO_3^-]_{BL}$ of 22 mM and a fixed pH_{BL} of 7.40. For *Top* and *Middle*, each of the 25 experiments paired an equilibrated bath CO_2/HCO_3^- solution (▲) with one of four OOE solutions (■). $n = 6$, $[CO_2]_{BL}$ values of 0, 2.5, and 10%; $n = 7$, $[CO_2]_{BL} = 20\%$. For *Bottom*, each of the 20 pH_i experiments included bath exposures to the equilibrated and one or two OOE solutions. $n = 12$, $[CO_2]_{BL} = 0$; $n = 6$, $[CO_2]_{BL} = 2.5\%$; $n = 8$, $[CO_2]_{BL}$ values of 10% and 20%. (*C*) Effect of changing pH_{BL} at a fixed $[CO_2]_{BL}$ of 5% and a fixed $[HCO_3^-]_{BL}$ of 22 mM. For *Top* and *Middle*, each of the 12 experiments paired an equilibrated bath CO_2/HCO_3^- solution (▲) with one of two OOE solutions (♦). $n = 6$, pH_{BL} values of 6.80 and 8.00. For *Bottom*, each pH_i experiment included bath exposures to each of the three solutions in *Top* ($n = 6$). *, $P < 0.01$; **, $P < 0.001$; absence of * or ** indicates $P > 0.05$ in paired two-tailed *t* tests comparing OOE data with equilibrated data.

Measurement of pH_i. We calculated pH_i from the fluorescence excitation ratio of 2′,7′-bis-(2-carboxyethyl)-5(and-6)carboxyfluorescein (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×/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 pH_i values by using the high-K^+/nigericin technique (25), as modified for one-point calibrations (26).

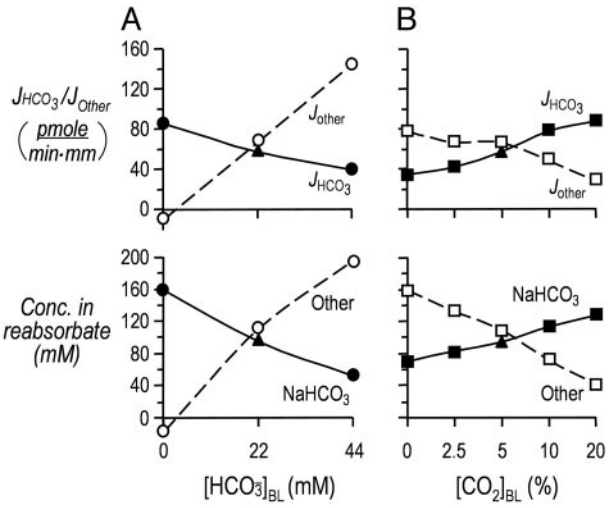

Fig. 2. Effect of changes in basolateral $[HCO_3^-]$ or $[CO_2]$ on the calculated reabsorption of solutes other than $NaHCO_3$. (*A*) Changes in $[HCO_3^-]_{BL}$. (*A Upper*) the J_{HCO_3} data are replotted from Fig. 1*A Top*. The J_{Other} values were calculated as described in *Materials and Methods*, assuming $(J_{HCO_3} + J_{Other})/J_V = 300$ mosM. (*A Lower*) the calculated reabsorbate $[NaHCO_3]$ values were obtained by dividing J_{HCO_3} values in *A* by the corresponding J_V values in *B*. The calculated concentrations of all other solutes (Other) was obtained by dividing J_{Other} in *A* by the corresponding J_V in *B*. (*B*) Isolated changes in $[CO_2]_{BL}$. The J_{HCO_3} data in *Top* are replotted from Fig. 1*B Top*. We computed the other values as described for Fig. 2*A*.

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 $[HCO_3^-]_{BL}$. In the Fig. 1*A Top*, the triangle ($[HCO_3^-]_{BL} = 22$ mM) represents J_{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 HCO_3^--free, while holding $[CO_2]_{BL}$ and pH_{BL} at their physiological values, caused J_{HCO_3} to increase by ≈50%. This maneuver approximates the "metabolic" half of metabolic acidosis. Conversely, switching from the equilibrated 5% CO_2/22 mM HCO_3^- at pH 7.40 solution to an OOE bath solution with twice the normal $[HCO_3^-]_{BL}$, but with $[CO_2]_{BL}$ and pH_{BL} at their physiological values, caused J_{HCO_3} to fall by ≈30%. Thus, changes in $[HCO_3^-]_{BL}$ cause J_{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 $[HCO_3^-]$ in this reabsorbed fluid is the ratio J_{HCO_3}/

PHYSIOLOGY

J_V. Fig. 1*A Middle* shows that changes in $[HCO_3^-]_{BL}$ do not cause significant changes in J_V. Thus, J_{HCO_3}/J_V (i.e., $[HCO_3^-]$ in the reabsorbate) must vary considerably. In Fig. 1*A*, this calculated $[HCO_3^-]$ was 159 mM at a $[HCO_3^-]_{BL}$ of 0 mM (i.e., the reabsorbate was isotonic $NaHCO_3$). In Fig. 2*A Upper*, we replot the J_{HCO_3} data from Fig. 1*A Top* and also plot the flux of all Other solutes, as described in *Materials and Methods*. Fig. 2*A Lower* summarizes the computed reabsorbate values of $[NaHCO_3]$ and all Other solutes. The analysis in Fig. 2*A* illustrates that increasing $[HCO_3^-]_{BL}$ not only lowers J_{HCO_3} but reciprocally raises the reabsorption of other solutes (J_{Other}), the appropriate response for maintaining a constant J_V and, thus, a constant blood pressure.

Because extracellular acid-base disturbances generally cause pH_i to change (27), we examined how changes in $[HCO_3^-]_{BL}$ affect the steady-state pH_i of PT cells under the same conditions that prevailed for Fig. 1*A Top* and *Middle*. As shown in the Fig. 1*A Bottom*, an increase in $[HCO_3^-]_{BL}$ from 0 to 22 mM caused steady-state pH_i to increase by 0.32. However, further increasing $[HCO_3^-]_{BL}$ to 44 mM did not cause a statistically significant increase in steady-state pH_i.

Effect of Isolated Variations in $[CO_2]_{BL}$. In Fig. 1*B Top*, the triangle ($[CO_2]_{BL} = 5\%$) represents virtually the same conditions as the triangle in Fig. 1*A Top*. Switching from this equilibrated, physiological solution to one in which we increased $[CO_2]_{BL}$ to four times its physiological value, while holding $[HCO_3^-]_{BL}$ and pH_{BL} at their physiological values, caused J_{HCO_3} to rise by ≈50% This maneuver approximates the "respiratory" half of respiratory acidosis. If we instead exposed the PT to a nominally CO_2-free OOE bath solution, while holding $[HCO_3^-]_{BL}$ and pH_{BL} at their physiological values, J_{HCO_3} fell by 40% compared with normal. The small J_{HCO_3} observed in the nominal absence of basolateral CO_2 may be due, in part, to CO_2 from the lumen reaching the basolateral membrane. The midpoint of the J_{HCO_3} response to CO_2 is at a $[CO_2]_{BL}$ of ≈6%, which is somewhat above the physiological $[CO_2]$ of arterial blood and somewhat below that of the renal cortex (28, 29). Thus, isolated changes in $[CO_2]_{BL}$ cause J_{HCO_3} to change in a direction that would help the PT to respond appropriately to stabilize blood pH.

Fig. 1*B Middle* shows that isolated changes in $[CO_2]_{BL}$ tended to cause J_V to increase. However, none of the differences between the J_V values obtained in OOE solutions and the J_V value obtained in the equilibrated solution (solution 7) were statistically significant. As $[CO_2]_{BL}$ rose from 0% to 20%, the calculated reabsorbate $[NaHCO_3]$ rose from 70 mM (i.e., the isosmotic reabsorbate was ≈50% $NaHCO_3$) to 129 mM (i.e., the reabsorbate was mainly isotonic $NaHCO_3$). As summarized in Fig. 2*B*, increases in $[CO_2]_{BL}$ not only raise J_{HCO_3} and reabsorbate $[NaHCO_3]$ but also reciprocally lower J_{Other} and the total concentration of other solutes in the reabsorbate.

We also measured pH_i of the PT cells under the conditions of Fig. 1*B Top* and *Middle*. Fig. 1*B Bottom* shows that increases in $[CO_2]_{BL}$ cause graded decreases in steady-state pH_i. Thus, the data in Fig. 1*B* are consistent with the hypothesis that elevations in $[CO_2]_{BL}$ increase J_{HCO_3} indirectly by lowering pH_i. According to this hypothesis, the increased J_{HCO_3} that we observed when decreasing $[HCO_3^-]_{BL}$ (Fig. 1*A Top*) also would have been caused by the attendant decrease in pH_i (Fig. 1*A Bottom*).

Effect of Variations in pH_{BL}. If pH_i changes determine J_{HCO_3}, we should be able to increase J_{HCO_3} by lowering pH_i, even without changing $[HCO_3^-]_{BL}$ or $[CO_2]_{BL}$. In Fig. 1*C Top*, the triangle ($pH_{BL} = 7.40$) represents the same conditions as the triangle in Fig. 1*B Top*. Surprisingly, switching to either a pH-6.80 or pH-8.00 OOE bath solution, while holding $[HCO_3^-]_{BL}$ and $[CO_2]_{BL}$ at their physiological values, caused no change in either J_{HCO_3} (*Top*) or J_V (*Middle*). As pH_{BL} rose from 6.80 to 8.00, the calculated $[HCO_3^-]$ in the reabsorbate ranged between 96 mM and 99 mM. On the other hand, Fig. 1*C Middle* shows that raising pH_{BL} from 6.80 to 8.00 caused substantial, graded increases in steady-state pH_i. In fact, the $\Delta pH_i/\Delta pH_o$ ratio for these PT cells (≈60%) is among the highest recorded for any cell (30–33).

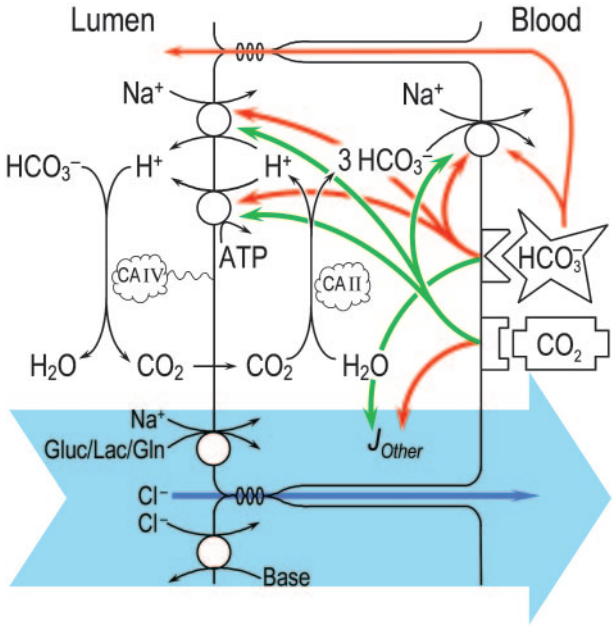

Fig. 3. Model of HCO_3^- reabsorption by the proximal tubule. Changes in pH_{BL} cause large changes in pH_i in the same direction but have no short-term effect on J_{HCO_3}. Increases in $[HCO_3^-]_{BL}$ could moderate J_{HCO_3} (red arrows) by increasing HCO_3^- backleak into the lumen and/or by directly inhibiting the electrogenic Na/HCO_3 cotransporter NBCe1-A. A HCO_3^- sensor would contribute to the decrease in J_{HCO_3} (red arrows) and is necessary to account for the increased reabsorption (green arrow) of solutes other than $NaHCO_3$ (J_{Other}). At the apical membrane, this reabsorption of other solutes (enclosed by the large blue arrow) would be mediated by cotransporters mediating the uptake of Na/glucose, Na/lactate, and Na/glutamine; obligated Cl^- would pass through tight junctions by diffusion and solvent drag. Apical Cl-base exchange (in parallel with Na-H exchange) could also contribute to J_{Other}. Increases in CO_2 would increase J_{HCO_3} by stimulating a CO_2 sensor at or near the basolateral membrane. This sensor would, in turn, stimulate three acid-base transporters (green arrows) but reduce J_{Other} (red arrow). If the CO_2 sensor is a membrane protein, the CO_2 binding site is not necessarily facing outward as shown.

Discussion

What Contributes to J_{Other}? One of our most striking observations is that J_{HCO_3} and J_{Other} (i.e., reabsorption rate of solutes other than HCO_3^- and its obligated Na^+) change reciprocally to keep J_V relatively constant. The Other solutes include (*i*) Cl^-, (*ii*) organic solutes, and (*iii*) Na^+ in excess of that accompanying HCO_3^-. Diffusion and solvent drag through tight junctions, possibly augmented by apical Cl-base exchange (34), could contribute to Cl^- reabsorption (Fig. 3). Apical cotransport of Na^+ with glucose, lactate, and glutamine could contribute to the reabsorption of organics. The near constancy of J_V implies that J_{Na} and, thus, the Na-K pump rate, must also be relatively stable.

The PT could produce the highest J_{Other} observed in the present study (≈143 pmol·min^{-1}·mm^{-1} in Fig. 2*A*) 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^+ and

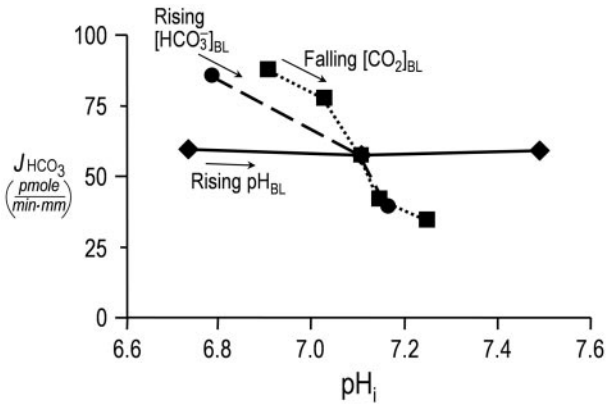

Fig. 4. Relationship between J_{HCO_3} and pH_i. The circles are replots of the J_{HCO_3} and pH_i data from Fig. 1*A Top* and *Bottom*, $[HCO_3^-]_{BL}$ values ranging from 0 mM on the left to 44 mM on the right (fixed $[CO_2]_{BL}$ = 5%, fixed pH_{BL} = 7.40). The squares are replots of data from Fig. 1*B*, $[CO_2]_{BL}$ values ranging from 20% on the left to 0% on the right (fixed $[HCO_3^-]_{BL}$ = 22 mM, fixed pH_{BL} = 7.40). The diamonds are replots of data from Fig. 1*C*, pH_{BL} values ranging from 6.80 on the left to 8.00 on the right (fixed $[CO_2]_{BL}$ = 5%, fixed $[HCO_3^-]_{BL}$ = 22 mM).

obligated Cl^-. To the extent that organic-solute transport contributes to J_{Other}, PT cells could reciprocally alter J_{HCO_3} and J_{Other} by changing the rate of the apical Na-H exchanger, predominantly NHE3 (35). To the extent that apical Cl-base exchange in parallel with Na-H exchange contributes to J_{Other}, the cell could reciprocally alter J_{HCO_3} and J_{Other} by changing $J_{Cl\text{-}base}$ while keeping $J_{Na\text{-}H}$ constant.

Do Proximal Tubules Sense Acute pH_i 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_i changes (36). Although the evidence is consistent with a major role for pH_i 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 J_{HCO_3} data from Fig. 1*A Top* vs. the corresponding pH_i data from Fig. 1*A Bottom*. Similarly, the squares are replots of the J_{HCO_3} vs. the pH_i data from Fig. 1*B*. For both data sets, increases in pH_i, whether caused by a rising $[HCO_3^-]_{BL}$ or a falling $[CO_2]_{BL}$, are associated with decreases in J_{HCO_3}. However, the replot of the J_{HCO_3} vs. pH_i data from Fig. 1*C*, plotted as diamonds in Fig. 4, shows that even large pH_i changes are not sufficient to alter J_{HCO_3}, provided both $[HCO_3^-]_{BL}$ and $[CO_2]_{BL}$ are constant. *Supporting Text* shows that large pH_i changes are likewise insufficient to alter J_{Other} (Fig. 5). Thus, pH_i cannot be the parameter triggering reciprocal changes in J_{HCO_3} and J_{Other} in Fig. 2 *A* and *B*.

In retrospect, the insensitivity of J_{HCO_3} to acute pH_i changes could have been anticipated for both theoretical and experimental reasons. First, theory predicts that a fall in pH_i could trigger an increase in J_{HCO_3} only by stimulating both H^+ extrusion across the apical membrane and HCO_3^- exit across the basolateral membrane. However, a fall in pH_i ought to inhibit NBCe1-A (Fig. 3), either directly or indirectly by lowering $[HCO_3^-]_i$ and $[CO_3^{2-}]_i$, just as a fall in pH_i inhibits Cl-HCO_3 exchange in other cell types (37–40).

Second, previous experiments showed that, with CO_2/HCO_3^- absent from both lumen and bath or present only in the lumen, pH_i recovers (i.e., increases) slowly from acute intracellular acid loads (41). Just as important, the pH_i recovery rates are only modestly pH_i sensitive. On the other hand, with CO_2/HCO_3^- present both in lumen and bath or only in the bath, pH_i recovers far more rapidly but still with only modest pH_i sensitivity (see fig. 4 in ref. 41). In other experiments (42), in which CO_2/HCO_3^- was either absent from both lumen and bath (bilateral Hepes) or present in both lumen and bath (bilateral CO_2/HCO_3^-), a more quantitative analysis of acid-extrusion rates (i.e., the sum of apical Na-H exchange and H^+ pumping) was possible. In bilateral Hepes, acid extrusion was low and only modestly pH_i dependent. In bilateral CO_2/HCO_3^-, acid extrusion was again only modestly pH_i dependent but ≈8-fold higher at identical pH_i values (see fig. 5 in ref. 42). Thus, the main determinant of acid extrusion in intact PTs is not pH_i but parameters related to $[HCO_3^-]_{BL}$ and/or $[CO_2]_{BL}$.

What, then, is the primary trigger for reciprocal changes in J_{HCO_3} and J_{Other}? A fall in $[HCO_3^-]_{BL}$ could lower HCO_3^- backleak through tight junctions (43, 44) and, thereby, raise J_{HCO_3}. NBCe1-A (Fig. 3) appears to transport CO_3^{2-} when operating with a Na^+:HCO_3^- stoichiometry of 1:2 (I. Grichtchenko and W.F.B., unpublished data) and may well transport both CO_3^{2-} and HCO_3^- when operating with a 1:3 stoichiometry in the PT. Thus, increases in $[HCO_3^-]_i$ and $[CO_3^{2-}]_i$ or decreases in $[HCO_3^-]_{BL}$ and $[CO_3^{2-}]_{BL}$ could stimulate NBCe1-A and thereby raise J_{HCO_3}. However, although effects on HCO_3^- backleak or NBCe1-A might serve as secondary modulators of J_{HCO_3}, it is unclear how these effects could serve as primary triggers for changes in J_{Other}, let alone closely coordinated, reciprocal changes in J_{HCO_3} and J_{Other} (Fig. 2 *Upper*).

Even though our data indicate that PT cells do not respond to acute pH_i changes by modulating J_{HCO_3} or J_{Other}, our data do not address the issue of how prolonged pH_i changes might affect cells. In opossum-kidney cells, chronic metabolic or respiratory acidosis (pH_o 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.

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 $[CO_2]$, pH, $[H_2CO_3]$, $[HCO_3^-]$, and $[CO_3^{2-}]$ (Fig. 6). The analysis reveals that no single parameter exhibits a pattern that consistently correlates, positively or negatively, with the patterns of J_{HCO_3} and J_{Other} in Fig. 2 *Upper*. Moreover, the analysis reveals that the next most parsimonious hypothesis, that the patterns of J_{HCO_3} and J_{Other} in Fig. 2 *Upper* reflect the sensing of two parameters, is straightforward for only three of 45 parameter pairs: (*i*) $[HCO_3^-]_{BL}$ and $[CO_2]_{BL}$, (*ii*) $[HCO_3^-]_{BL}$ and $[CO_2]_i$, and (*iii*) $[HCO_3^-]_{BL}$ and $[H_2CO_3]_i$ (Table 2). As summarized in Fig. 3, we propose that PT cells respond to increases in $[HCO_3^-]_{BL}$ by reducing J_{HCO_3} and raising J_{Other} and respond to increases in $[CO_2]_{BL}$ (or a related parameter) by raising J_{HCO_3} and reducing J_{Other}.

We already noted that adding CO_2/HCO_3^- 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 CO_2 strongly enhanced acid-base transporters, overwhelming the inhibition imposed by the basolateral HCO_3^-. Because adding CO_2/HCO_3^- to the lumen fails to stimulate acid extrusion (41), we propose that CO_2 stimulates a sensor at or near the basolateral membrane. In principle, the sensor could face the basolateral fluid and sense $[CO_2]_{BL}$ *per se*, or the sensor could be inside the cell near its basolateral membrane and sense $[CO_2]_i$ or $[H_2CO_3]_i$.

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

PHYSIOLOGY

determine pH: HCO_3^- and CO_2. Moreover, it appears that the HCO_3^- and CO_2 sensors operate in a push–pull fashion to diminish J_{HCO_3} and augment J_{Other} in the case of HCO_3^- and to augment J_{HCO_3} but diminish J_{Other} in the case of CO_2. The net effects are not only to trigger changes in J_{HCO_3} that tend to restore blood pH but also to trigger compensatory alterations in the reabsorption of other solutes, thereby stabilizing J_V. Understanding how the PT transduces the HCO_3^- and CO_2 signals may provide important clues for treating both acid-base disturbances and hypertension.

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