Regulation of Intracellular pH in the Rabbit Cortical Collecting Tubule

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Abstract

The cortical collecting tubule (CCT) is an important nephron segment for Na⁺, K⁺, water and acid-base transport. Differential loading characteristics of the pH sensitive dye 2',7'-bis-(2-carboxyethyl)-5(and-6)carboxyfluorescein (BCECF) and basolateral Cl⁻ removal were used to identify and study intracellular pH (pHi) regulation in each of three cell types involved in this transport. Both principal cells and β-intercalated cells were found to have a basolateral Na⁺/H⁺ exchanger based on the Na⁺ and amiloride sensitivity of pHi recovery from acid loads.

Intercalated cells demonstrated abrupt pHi changes with basolateral Cl⁻ removal. α-intercalated cells alkalized; β-intercalated cells acidified. In the β-intercalated cells, luminal Cl⁻ removal blocked changes in pHi, in response to changes in luminal HCO₃⁻ or peritubular Cl⁻, providing direct evidence for a luminal Cl⁻/HCO₃⁻ exchanger. In principal cells, brief removal of either peritubular or luminal Cl⁻ resulted in no change in pHi; however, return of peritubular Cl⁻ after prolonged removal resulted in a rapid fall in pHi consistent with a basolateral Cl⁻/HCO₃⁻ exchanger, which may be relatively inactive under baseline conditions. Therefore, Cl⁻/HCO₃⁻ exchange is present in all three cell types but varies in location and activity. (J. Clin. Invest. 1990. 85:274-281.) 2',7'-bis-(2-carboxyethyl)-5(and-6)carboxyfluorescein (BCECF) • Cl⁻/HCO₃⁻ exchange • intercalated cell • Na⁺/H⁺ exchange • principal cell

Introduction

The collecting tubule of the mammalian nephron is the segment of final modulation of urinary excretion of Na⁺, K⁺, water, and acid-base equivalents. In the cortical collecting tubule (CCT) regulation of the excretion or reabsorption of each of these occurs. Corresponding with the diversity of functions, the CCT is a heterogenous tissue composed of multiple cell types: principal cells and at least two types of intercalated cells (1, 2).

Principal cells are the most numerous cell type and appear to function primarily to reabsorb Na⁺, secrete K⁺, and modulate arginine-vasopressin induced water reabsorption (3, 4). Principal cell pHi regulation may be important in the regulation of salt and water transport by this cell. Intracellular acidosis appears to decrease Na⁺ transport in the CCT and analogous epithelia (5–9). In addition, pHi may alter the hydroosmotic response to antidiuretic hormone (ADH) and cAMP (10–12). Conversely, ADH appears to activate numerous acid-base transporters in both renal (13, 14) and nonrenal tissues (15, 16). ADH may also have effects on urinary acidification (17).

Intercalated cells are interspersed between principal cells, make up ~ 35–40% of the total cells of the CCT (1, 2), and both secrete (18) and reabsorb HCO₃⁻ (19). At least two subtypes of intercalated cells are present. The α-intercalated cell secretes H⁺ and reabsorbs luminal HCO₃⁻. An electronegatic, H⁺ translocating ATPase is present at the luminal membrane (20). HCO₃⁻ exits the cell via a basolateral Cl⁻/HCO₃⁻ exchanger. The Cl⁻/HCO₃⁻ exchanger is sensitive to the disulfonic stilbenes (21) and is immunologically similar to the Cl⁻/HCO₃⁻ exchanger of the mammalian red blood cell, band 3 protein (22, 23).

The second type of intercalated cell is the β-intercalated cell. The β-intercalated cell is postulated to be responsible for HCO₃⁻ secretion by the CCT. This transport is modeled to occur via a luminal Cl⁻/HCO₃⁻ exchanger and a basolateral H⁺-ATPase. This cellular model has been derived in large part by indirect inferences from transepithelial flux measurements. The basolateral location of H⁺-ATPase has been confirmed by immunocytochemical studies (20). However, direct confirmation of the luminal Cl⁻/HCO₃⁻ exchanger has been difficult. Antibodies to band 3 protein do not stain the apical membrane (22, 23) and luminal disulfonic stilbenes do not inhibit bicarbonate secretion (21, 24). As a result, direct confirmation of the luminal Cl⁻/HCO₃⁻ exchanger in the β-intercalated cell has been lacking.

The purpose of the present studies was to characterize the distribution of the major mechanisms of pHi regulation in two of the cell types (principal cell and β-intercalated cell) present in the rabbit CCT. Evaluation of the mechanisms of pHi regulation in the CCT has been difficult due to the cellular heterogeneity of this segment of the nephron. We have recently described a technique whereby principal cell and intercalated cell pHi can be separately measured in the in vitro, microperfused rabbit CCT using the fluorescent, pH sensitive dye 2',7'-bis-(2-carboxyethyl)-5(and-6)carboxyfluorescein (BCECF) (25). Therefore, the specific purpose of these studies was to characterize the distribution of Na⁺/H⁺ and Cl⁻/HCO₃⁻ exchangers in the rabbit CCT.

Methods

Microperfusion. In vitro microperfusion of cortical collecting tubules was performed using standard techniques (26). Dissection of individ-

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1. Abbreviations used in this paper: ADH, antidiuretic hormone; BCECF, 2',7'-bis-(2-carboxyethyl)-5(and-6)carboxyfluorescein; BCECF-AM, acetoxymethyl ester of BCECF; CCT, cortical collecting tubule; pHi, intracellular pH.

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usual CCT was performed in cold solution 1 (for studies with CO2 containing solutions) or in solution 4 (for studies with CO2 free solutions) (see Table I) containing 5% fetal calf serum. Tubules were studied in a 1-mi chamber thermostatically controlled to 37°C; the peritubular bathing solution was continuously exchanged at a rate of ~ 3 ml/min. CO2-impermeable Saran tubing (Clarkson Equipment & Controls, Detroit, MI) was used to deliver the bathing solution. Bath pH was continuously monitored with a flexible pH electrode (model MI-508; Microelectrodes, Inc., Londonderry, NH).

**Solutions.** The components of the various solutions used in the study are shown in Table I. Solutions 1–3, 6–8 were used for experiments performed in the presence of CO2; solutions 4 and 5 were used for experiments performed in the nominal absence of CO2. CO2 containing solutions were bubbled with 95% O2/5% CO2. CO2-free solutions were bubbled with 100% O2. CO2-free solutions were adjusted to pH 7.40 with tetramethylammonium hydroxide instead of sodium hydroxide in order to minimize the Na+ concentration of Na+ free solutions. Total Ca2+ was increased in solutions 3, 7, and 8 to compensate for complexing of Ca2+ by gluconate. All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise specified.

**Fluorescent dyes.** The acetoxyethyl ester of BCECF (BCECF-AM), was obtained from Molecular Probes, Inc. (Eugene, OR) and maintained at ~20°C as a 30-mM stock solution in DMSO. On the day of an experiment 15 μM (for luminal loading, see below) or 5 μM (for basolateral loading, see below) solutions were made by diluting with either solution 1 (for CO2/HCO3 containing experiments) or solution 4 (for CO2/HCO3 free experiments).

Loading with BCECF was performed as previously described (25). In brief, luminal BCECF-AM is selectively concentrated by intercalated cells, while peritubular BCECF-AM is homogeneously taken up by both principal and intercalated cells (25). As a result, intercalated cells were studied after loading with luminal BCECF-AM. Principal cells were studied by loading first with luminal BCECF-AM, identifying an area of the tubule without intercalated cells, and then loading with peritubular BCECF-AM. In all cases at least 5 min was allowed after loading BCECF-AM before measurement of pHi.

**Intracellular pH measurements.** Fluorescence studies were performed on a Nikon Diaphot-TMD microscope modified for fluorescent use as previously described (25). pHi measurements were made by exciting an area of ~ 5 μm diam. In general this field was positioned at the edge of the tubule to minimize fluorescence from cells above or below the plane of measurement. Use of a small excitation field centered at the edge of the tubule thereby allowed measurement of the pHi of either a single intercalated cell or portions of approximately one to four principal cells. pHi measurements were made by alternatively exciting at 500 and 450 nm. Fluorescence at 530 nm was measured by a Nikon P1 photometer. The analog output from the photometer was digitized (8232; Starbase Data Co., Salt Lake City, UT) and recorded on a personal computer for analysis at a later time. Background fluorescence was < 5% of dye fluorescence at both excitation wavelengths and was subtracted before calculation of fluorescence ratio. pHi results were graphed using a moving 10–15-s time average technique. These tracings were then used for measurement of pHi for analysis. Although amiloride is a fluorescent molecule, 1 mM peritubular amiloride had no effect on fluorescent ratio in cells which were clamped at a fixed pHi, using the high-K ′/Nigericin technique of Thomas et al. (27) (results not shown).

Changes in pHi after acid loading are expressed (except where specifically noted) as the difference in pHi between 1 and 5 min after the solution change; the initial 1-min time point was chosen to match the nadir of pHi after acid loading and to ensure complete solution change. A change in the luminal fluid required a period of ~ 1–3 min during which pHi was not measured. After completion of the perfusate change, pHi was measured for at least 5 min or until it had stabilized. The pHi at the end of this period was used for analysis.  

**Calibration.** Calibration of intracellular BCECF was performed using the method of Thomas et al. (27). The calibration solution contained (in mM) 120 KCl, 1.2 CaCl2, 1.0 MgCl2, 2.0 NaH2PO4, 25 Hepes, and 14 μM nigericin and was adjusted to pH 6.8, 7.0, 7.2, 7.4, 7.6, and 7.8 using NaOH and HCl. Least-squares, linear regression was performed to fit the calibration points to the equation: ratio (500/450nm) = a + b·pHi. The calibration curve was then used for conversion of calculated fluorescent ratio to pHi. Separate calibration curves were performed for intercalated cells and principal cells. In most experiments a calibration was performed for the cell type studied in that experiment and used for conversion of calculated ratio to pHi; in a few experiments mean calibration curves from prior experiments were used for conversion. Dye concentration during calibration, as measured by fluorescence at 450 nm excitation, was similar to that during experimental pHi measurements.

**Acid loading.** Acid loading was achieved by changing the peritubular solution to one containing 10 mM NH4Cl (for principal cells and some β-intercalated cells) or 20 mM NH4Cl (for some β-intercalated

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**Table I. Solutions**

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* All concentrations expressed in millimoles per liter. Osmolality adjusted to 285–295 mosmol/kg by addition of the major salt. Solutions 4 and 5 bubbled with 100% O2, all others with 95% O2/5% CO2.
cells) for a period of 5 min; removal of NH4Cl resulted in an abrupt acidification. Equimolar amounts of the principal salt in the NH4Cl containing solutions were removed so that solution osmolality remained constant. Although 20 mM NH4Cl resulted in slightly greater acidification, no qualitative differences in results were seen.

Statistics. Values are presented as mean±SEM. In general, statistical tests are performed using paired, two sided Student’s t test. Unpaired, two-sided Student’s t tests were used when appropriate and are noted in the text. Statistical significance is defined as at least P < 0.05. All pHi results are reported as the mean of n tubules.

Results

Principal cell pHi. Baseline pHi for principal cells was obtained in tubules bathed and perfused with identical solutions for at least 30 min before any measurements. The baseline pHi in CO2-containing solutions (solution 1) was 7.36±0.05 (n = 13), while in the absence of CO2 (solution 2) the baseline pHi was significantly higher at 7.77±0.06 (n = 13) (P < 0.001 by unpaired t test).

Principal cell Na+/H+ exchange. The importance of Na+/H+ exchange on principal cell pHi recovery after an acid load was investigated. All experiments evaluating principal cell Na+/H+ exchange were performed in CO2/HCO3 free solutions in order to minimize the effect of HCO3 transporters on pHi regulation. A 5-min application and then removal of 10 mM ammonium chloride (solution 5 plus NH4Cl) was used to acidify principal cells to a mean pHi of 6.84±0.09 (n = 10). After 5 min in a Na+ free peritubular solution (solution 5) principal cell pHi increased by only 0.16±0.11 pH U (n = 6, NS from 0.00). By contrast, after 5 min in a Na+ containing peritubular solution (solution 4), pHi in the same principal cells increased 0.50±0.05 pH U (n = 6, P < 0.05 vs. Na+ free). These results show that principal cells possess a Na+ dependent mechanism for pHi regulation after an acid load. (In most experiments the peritubular bathing solution was changed first to a Na+ free solution for 5 min and then to the Na+ containing peritubular solution; the principal cells were generally at the same, or occasionally slightly higher, initial pHi on exposure to the Na+ containing solution. Since the Na+/H+ exchanger has been well characterized to be inhibited by an increased pHi, the increased rate of recovery of pHi during Na+ containing basolateral solutions cannot be explained by the difference in the initial pHi.)

To confirm this Na+ dependent pHi recovery as Na+/H+ exchange, the inhibitory effect of 1 mM amiloride on pHi recovery after an acid load was investigated. A typical experiment is shown in Fig. 1. In the presence of peritubular amiloride, the pHi recovery after 5 min was 0.13±0.05 pH U, while in the absence of amiloride pHi recovery in the same cells was 0.67±0.08 (n = 7, P < 0.005 by paired t test). These results suggest that a basolateral, amiloride inhibitable, Na+-dependent mechanism, i.e., Na+/H+ exchange, is a major mechanism for principal cell recovery from an acid load.

To determine if Na+/H+ exchange is active at baseline pHi, the effect of Na+ removal on principal cell pHi was investigated. Removal of Na+ from the peritubular solution (changing to solution 5) resulted in a slow acidification of 0.10±0.04 pH U (n = 8, P < 0.05) over a 5-min period.

Cl− and principal cell pHi. Cl− dependent pHi regulatory processes appear to be present in most cell types (28); the presence of these was investigated next. Removal of Cl− from CO2/HCO3 containing luminal solutions (changing the perfusate from solution 1 to solution 3) for 5–10 min resulted in no significant change in principal cell pHi (difference −0.02±0.03, P = NS, n = 4). Similarly, return of Cl− to the luminal fluid (change back to solution 1) resulted in no significant change in pHi (difference −0.01±0.04, P = NS, n = 4). Evidence for a basolateral Cl− linked pHi regulatory mechanism was then studied. A 5-min removal and then return of Cl− to the peritubular solution (change from solution 1 to solution 3 and then return to solution 1) resulted in no significant changes in principal cell pHi (differences −0.02±0.03 and −0.06±0.03, for removal and return of peritubular Cl−, respectively, n = 5, P = NS for both). However, since intracellular Cl− concentrations in principal cells are quite low, ~7–14 mmol/kg dry wt in the rat (29, 30) and principal cells possess a basolateral Cl− channel (31), removal of peritubular Cl− may result in gradients for Cl−/HCO3 exchange that are relatively small, possibly explaining the relative absence of pHi changes after 5 min of peritubular Cl− removal. A prolonged removal of peritubular Cl− might result in intracellular Cl− depletion and an increased gradient for Cl− entry during return of Cl− to the peritubular solution. A 30-min incubation in a Cl− free peritubular solution (solution 3) resulted in a slow increase in pHi from 7.35±0.08 to a maximum of 7.55±0.10 (P < 0.005, n = 5). After return of peritubular Cl− (change back to solution 1) pHi fell rapidly. 5 min after the change pHi had fallen by 0.30±0.04 pH U (P < 0.005 by paired t test, n = 5). These results are consistent with the exchange of intracellular HCO3 for extracellular Cl−, i.e., basolateral Cl−/HCO3 exchange activity, during the return of basolateral Cl− to Cl− depleted principal cells.

Intercalated cell-baseline pHi, and CO2/HCO3, β-intercalated cell baseline pHi was measured in the presence and absence of CO2/HCO3. In the presence of CO2/HCO3 (luminal and peritubular solution 1) the baseline pHi was 7.33±0.04 (n = 32). In the nominal absence of HCO3/CO2 (luminal and peritubular solution 4), β-intercalated cell pHi was 8.05±0.02 (n = 4, P < 0.001 vs. pHi in the presence of CO2/HCO3 by unpaired t test). However, BCECF may not reliably reflect true pHi at such extremes of pH. Consistent with this is the finding
that in the nominal absence of CO₂ and HCO₃⁻ no change was seen in β-intercalated cell apparent pH, with either addition or removal of basolateral NH₄⁺ (data not shown). Therefore, all subsequent intercalated cells experiments were performed with CO₂ present in the both the peritubular and luminal solutions.

**Intercalated cells, identification of subtype by Cl⁻ removal.** Basolateral Cl⁻ removal resulted in two distinct patterns of pHₜ response in intercalated cells. Occasional cells underwent a rapid alkalization from pHₜ 7.17±0.07 to 7.64±0.05 (n = 5, P < 0.001) after basolateral Cl⁻ removal. This was completely reversible with return of Cl⁻ to the peritubular solution, consistent with the presence of a basolateral Cl⁻/HCO₃⁻ exchanger. A representative experiment is shown in Fig. 2. These cells were infrequently encountered and further data will not be presented.

The majority of intercalated cells underwent a rapid intracellular acidification after basolateral Cl⁻ removal, functionally identifying it as the β-intercalated cell. The response of intercalated cells to basolateral Cl⁻ removal allows for a functional differentiation of the two types of intercalated cells. In all experiments on β-intercalated cells, sub-type was confirmed by intracellular acidosis in response to basolateral Cl⁻ removal.

In β-intercalated cells with basolateral Cl⁻ removal, the average decrease in pHₜ was −0.70±0.04 pH U in 3.9±0.04 min (n = 28, P < 0.001). Return of basolateral Cl⁻ resulted in the return of pHₜ to baseline. This has been reported by others and hypothesized to result from acceleration of apical Cl⁻/HCO₃⁻ exchange (32). To examine this hypothesis the effects of luminal Cl⁻ and HCO₃⁻ on pHₜ was examined. Nominal removal of HCO₃⁻ from the luminal solution (change to solution 6) resulted in a decrease of 0.41±0.05 pH U (P < 0.005, n = 8). Luminal Cl⁻ removal (change to solution 3) caused pHₜ to increase 0.45±0.07 pH U (n = 7, P < 0.005). Furthermore, the removal of luminal Cl⁻ blocked the effect of basolateral Cl⁻ removal on intracellular pH (see Fig. 3). Removal of basolateral Cl⁻ (by changing to solution 3) in the absence of luminal Cl⁻ resulted in no significant change in pHₜ (−0.11±0.07, n = 6, P = NS). The subsequent return of luminal Cl⁻ in the absence of basolateral Cl⁻ resulted in a marked acidification (ΔpHₜ = −0.73±0.18, n = 6, P < 0.01). This pHₜ is not significantly different than the pHₜ present after basolateral Cl⁻ removal (P > 0.3, unpaired t test), consistent with changes in pHₜ via identical mechanisms.

One likely explanation for the decrease in pHₜ with luminal HCO₃⁻ removal is the exchange of intracellular HCO₃⁻ for luminal Cl⁻ via an apical Cl⁻/HCO₃⁻ exchanger. Therefore the effect of luminal Cl⁻ on β-intercalated cell response to a decrease in luminal HCO₃⁻ concentration was evaluated. Glucose was substituted for the removed HCO₃⁻ so that luminal Cl⁻ would remain constant. In the presence of luminal Cl⁻, decreasing perfusate HCO₃⁻ from 25 to 5 meq/liter (changing from solution 1 to 7) caused pHₜ to decrease 0.24±0.06 pH U (P < 0.025, n = 4). In the absence of luminal Cl⁻ there was no significant change in pHₜ (change from solution 3 to 8, Δ = +0.05±0.03, P = NS vs. 0.00, P < 0.01 vs. changes in the presence of luminal Cl⁻ by paired t test, n = 4). In additional experiments to test this model, tubules were bathed and perfused with a high K⁺ solution containing 5 μM valinomycin in order to voltage clamp both the apical and basolateral membranes. Removal of luminal Cl⁻ caused pHₜ to reversibly increase 0.20±0.05 pH U [n = 2]. This is consistent with an apical, electroneutral, Cl⁻ dependent, base exit process, i.e., Cl⁻/HCO₃⁻ exchange. However, perfusion of CCT with high K⁺ solutions results in marked cell swelling [33, 34, and personal observations] and hence this method was not pursued further.

These results suggest that Cl⁻/base exchange at the apical membrane, in series with a basolateral Cl⁻ transport mechanism, most likely a Cl⁻ channel (35), is present in the β-intercalated cell.

**β-intercalated cell Na⁺/H⁺ exchange.** β-Intercalated cells were examined for the presence or absence of basolateral Na⁺/H⁺ exchange activity. The ammonium chloride pulse technique was used to acid load the cells to a mean pHₜ of 6.77±0.07 (n = 16). A typical experiment is shown in Fig. 4. In a Na⁺ free perfusate solution (solution 2) pHₜ recovered 0.08±0.04 pH U (n = 11) after 5 min. When Na⁺ was returned to the peritubular solution pHₜ recovery was markedly increased to 0.38±0.07 pH U (n = 11, P < 0.001 by paired t test).

The inhibitory effect of basolateral amiloride on pHₜ recovery of β-intercalated cells was then investigated. A concentration of 1 mM amiloride was used (in solution 1) in order to achieve a high degree of inhibition of the Na⁺/H⁺ exchange (36). Results are summarized in Fig. 5. After intracellular acid loading, the pHₜ recovery at 5 min in the presence of amiloride was 0.05±0.04, while in the absence of amiloride the recovery was 0.37±0.05 pH U (n = 5, P < 0.025). β-intercalated cell recovery from an acid load is via a basolateral Na⁺ dependent, amiloride inhibitable mechanism, i.e., Na⁺/H⁺ exchange.
Mechanism of \( p_{\text{H}} \) recovery in \( \beta \)-intercalated cells after basolateral Cl\(^-\) removal. Basolateral Cl\(^-\) removal from \( \beta \)-intercalated cells results in a marked acidification as described above. These intercalated cells possess multiple acid-base transporters (basolateral Na\(^+\)/H\(^+\) exchange, luminal Cl\(^-\)/HCO\(_3\)\(^-\) exchange, and basolateral H\(^+\)-ATPase; see introduction) which may participate in regulation of \( p_{\text{H}} \). Yet in the continued absence of basolateral Cl\(^-\) for up to 10 min, no significant recovery of \( p_{\text{H}} \) occurred (data not shown), despite the presence of basolateral Na\(^+\). To examine the activity of basolateral Na\(^+\)/H\(^+\) during the absence of basolateral Cl\(^-\) (and therefore a low \( p_{\text{H}} \)), the effect of amiloride was studied. Peritubular amiloride (1 mM dissolved in solution 3) resulted in a further decrease in \( p_{\text{H}} \) of 0.40±0.13 pH U (\( n = 7, P < 0.025 \)) after 5 min. This decrease was reversible with the removal of amiloride, suggesting that \( \beta \)-intercalated cell basolateral Na\(^+\)/H\(^+\) exchange is active during the absence of basolateral Cl\(^-\).

Discussion

These studies address the mechanisms of \( p_{\text{H}} \) regulation in the rabbit CCT. The three major cell types (principal cell, \( \alpha \)-intercalated cell, and \( \beta \)-intercalated cell) of this segment of the nephron were studied separately utilizing differences in BCECF loading characteristics (25) and response to peritubular Cl\(^-\) removal (32). The markedly different responses of the different cell types to peritubular Cl\(^-\) removal serve to substantiate our method of studying these cell types separately. The location of Na\(^+\)/H\(^+\) exchange and Cl\(^-\)/HCO\(_3\)\(^-\) exchange were studied in particular because of the apparent ubiquity of these transporters in mammalian cells.

The first major result of these studies is the demonstration that Na\(^+\)/H\(^+\) exchange is present at the basolateral membrane of both principal cells and \( \beta \)-intercalated cells. Principal cell \( p_{\text{H}} \) recovery after an acid load was Na\(^+\)-dependent and amiloride-inhibitable. The use of nominally CO\(_2\)/HCO\(_3\)\(^-\) free solutions minimizes the likelihood that Na\(^+\)-dependent HCO\(_3\)\(^-\) transport was responsible for the observed \( p_{\text{H}} \) changes. The most likely mechanism for an amiloride-sensitive, Na\(^+\)-dependent recovery from an acid load is a Na\(^+\)/H\(^+\) exchanger. Although not specifically tested, this exchanger is apparently present only on the basolateral membrane since little, if any, \( p_{\text{H}} \) recovery occurred with no peritubular Na\(^+\), even in the presence of luminal Na\(^+\).

A basolateral membrane Na\(^+\)/H\(^+\) exchanger was also identified in the \( \beta \)-intercalated cell. In the absence of basolateral Na\(^+\), almost no recovery of \( p_{\text{H}} \) occurred from ammonium chloride pulse-induced intracellular acidosis. Amiloride resulted in a similar inhibition of \( p_{\text{H}} \) recovery after an acid load. Since the studies on \( \beta \)-intercalated cells were performed in CO\(_2\)/HCO\(_3\)\(^-\) containing solutions, it is possible that the recovery was via HCO\(_3\)\(^-\) transporters. Multiple Na\(^+\)-linked HCO\(_3\)\(^-\) transporters, such as Na(HCO\(_3\))\(_2\) \( (37, 38) \) and Na\(^+\)-dependent Cl\(^-\)/HCO\(_3\)\(^-\) exchange (39), are known to exist. However, amiloride has not been shown to inhibit any of these (36). These results therefore provide the first evidence that basolateral Na\(^+\)/H\(^+\) exchange is present in the \( \beta \)-intercalated cell.

Na\(^+\)/H\(^+\) exchange is a major mechanism of \( p_{\text{H}} \) regulation after acid loads in almost every cell in which it has been studied (40). It also may be involved in growth factor signal transduction (41), cell volume regulation (42), and transepithelial Na\(^+\) transport (as in the proximal tubule, for example [43]). Boron et al. (44) has identified a Na\(^+\)/H\(^+\) exchanger on the basolateral membrane of the rabbit CCT. Although intercalated and principal cells could not be differentiated by their techniques, they concluded that Na\(^+\)/H\(^+\) exchange was present at the basolateral membrane of principal cells of the rabbit CCT because of the predominance of principal cells and because Na\(^+\)/H\(^+\) exchanger activity was seen in all experiments. In addition, they too found no evidence for luminal Na\(^+\)/H\(^+\) exchange activity in the CCT. The results of the present study extend their findings by demonstrating the presence of a basolateral Na\(^+\)/H\(^+\) exchanger in both principal cells and \( \beta \)-intercalated cells. Breyer et al. (45) and Hayes et al. (46) have demonstrated basolateral Na\(^+\)/H\(^+\) exchange in the inner stripe of the outer medullary collecting duct, which appears to be functionally equivalent to the \( \alpha \)-intercalated cell in the CCT. Na\(^+\)/H\(^+\) exchange has also been shown in papillary collecting tubule cells (47, 48). Therefore, all cells of the collecting tubule probably have basolateral Na\(^+\)/H\(^+\) exchange.

The finding that the major mechanism of \( p_{\text{H}} \) recovery in \( \beta \)-intercalated cells was Na\(^+\)/H\(^+\) exchange was initially quite surprising. These cells presumably have basolateral H\(^+\)-ATPase (20), and intracellular acidification may stimulate the insertion of additional H\(^+\)-ATPase from endosomes (49, 50). The inhibition of \( p_{\text{H}} \) recovery by the absence of basolateral Na\(^+\) or the presence of amiloride suggests that H\(^+\)-ATPase is not a major factor in acute \( p_{\text{H}} \) regulation in the \( \beta \)-intercalated cell. Several factors may account for the observation that Na\(^+\) independent processes (i.e., H\(^+\)-ATPase) apparently do not contribute significantly to acute \( p_{\text{H}} \) recovery. Membrane H\(^+\)-
ATPase activity appears to be regulated by insertion of H+ -
ATPase into and removal from the plasma membrane via
exocytosis and endocytosis, respectively (49-51). This process
might not allow H+ -ATPase to acutely regulate pHi. To the
extent that endocytosis and exocytosis of H+ -ATPase might
acutely regulate pHi, the intracellular alkalization during the
application of ammonium chloride could lead to endocytosis
of H+ from the basolateral membrane (51), thereby relatively
decreasing the potential H+ -ATPase-mediated pHi recovery
during the first 5 min of acidosis. Also possible is that this cell,
which functions to secrete base into the urine, might turn-off
the mechanisms of HCO3 secretion, e.g., H+ -ATPase, in re-
spone to intracellular acidosis. These considerations and the
results of this study suggest that a basolateral Na+/H+ ex-
changer participates in the regulation of β-intercalated cell
pHj; insertion of H+ -ATPase into the basolateral membrane of
the β-intercalated cell may be geared toward transepithelial
HCO3 transport and not acute pHj regulation.

Does the basolateral Na+/H+ exchanger in β-intercalated
cells have some role in CCT bicarbonate secretion? Recent
studies by Star et al. (52) found that bilateral (luminal and
basolateral) Na+ removal did not affect rabbit CCT HCO3
secretion. However, other studies have differed. McKinney
and Burg studied HCO3 secreting CCT and found that bicar-
sionate secretion was dependent on peritubular Na+ (18). Ar-
ruda et al. (53) showed in the turtle bladder (a model epithel-
ium for the CCT) that bilateral Na+ removal decreased HCO3
secretion. Further studies are necessary to delineate the role, if
any, of Na+/H+ exchange in CCT HCO3 secretion.

The other major class of acid-base transporters examined
in this study was the Cl-/HCO3 exchange process. Principal
cell Cl-/HCO3 exchange was carefully examined. Short-term
(5 min) removal of either luminal or basolateral Cl- did not
result in a significant change in pHj. A prolonged removal and
then return of basolateral Cl- was necessary to demonstrate
changes in principal cell pHj, consistent with the presence of a
basolateral Cl-/HCO3 exchanger, albeit relatively inactive.
This transporter clearly differs from that in the α- and β-inter-
calated cells. First, pHj in α- and β-intercalated cells responds
rapidly and dramatically to peritubular Cl- removal (see Figs.
2 and 3). Next, the principal cell Cl-/HCO3 exchanger does not
cross-react with antibodies to band 3 protein, as does the
Cl-/HCO3 exchanger of the α-intercalated cell (22, 23). Fi-
nally, it is located at the opposite membrane from the β-inter-
calated cell Cl-/HCO3 exchanger. The role of the principal cell
Cl-/HCO3 exchanger is not known, but it could function in
recovery from intracellular alkalosis, as Cl-/HCO3 exchangers
do in many other cell types (28, 45, 54). The minimal (and
slow) response to basolateral Cl- removal in principal cells is
consistent with relative inactivity of this transporter at baseline
pHj and confirms that the principal cell is not involved signifi-
cantly in transepithelial HCO3 transport.

In intercalated cells, basolateral Cl- removal resulted in
opposite pHj responses in two different populations. Schwartz
et al. (32) first showed this differential pHj response to baso-
lateral Cl- removal. In the present studies, the majority of in-
tercalated cells responded with a rapid intracellular acidification.
The less frequent intercalated cell responded to basolateral Cl-
removal with intracellular alkalization, consistent with the
presence of basolateral Cl-/HCO3 exchange. In view of the less
frequent occurrence of these intercalated cells and their alka-
linization in response to basolateral Cl- removal, they are
functionally identified as α-intercalated cells. Schwartz et al.
(55) has recently described a third type of intercalated cell;
these cells were infrequently found and appeared to have a
basolateral CI-/HCO3 exchanger. In the present study these
cells could have been identified as α-intercalated cells.

Luminal Cl-/HCO3 exchange was clearly present in β-in-
tercalated cells. The pHj decrease with basolateral CI- removal
in the β-intercalated cell was completely dependent on the
presence of luminal Cl-, suggesting that the removal of baso-
lateral Cl- affected a luminal, Cl- dependent transporter. Ba-
solateral Cl- removal probably induces intracellular Cl- exit
via a basolateral Cl- channel (3, 35), thereby resulting in an
increased lumen to cell Cl- gradient. The decrease in β-inter-
calated cell pHj, with basolateral Cl- removal is therefore con-
sistent with a luminal Cl-/HCO3 exchanger transporting Cl-
from the lumen into the cell in exchange for HCO3. Similarly,
the increase in pHj with luminal Cl- removal and the decrease
in pHj with luminal HCO3 removal are consistent with a
luminal Cl-/HCO3 exchanger. Inhibition of the fall in pHj, in re-
sponse to a decrease in luminal HCO3 by removal of luminal
Cl- shows that an apical Cl-/HCO3 exchanger is present at the
apical membrane of the β-intercalated cell. Studies by Schuster
and Stokes have suggested the presence of a luminal trans-
porter able to exchange CI- for either HCO3 or Cl- (56); this
transporter is stimulated by CO2/HCO3 (57). CCT HCO3 sec-
tion appears to involve the coupled, 1:1 reabsorption of CI-,
suggestive of a luminal Cl-/HCO3 exchanger being responsible
for HCO3 secretion (52). In summary, these results provide
direct functional evidence for luminal Cl- /base exchange,
most likely Cl-/HCO3 exchange, in series with basolateral Cl-
transport in the β intercalated cell type of the rabbit CCT.

An interesting finding in the β-intercalated cell is that no
pHj recovery occurred during the intracellular acidosis asso-
ciated with basolateral Cl- removal. Yet this was at a pHj at
which Na+/H+ exchange activity was clearly demonstrable
after NH4 pulse induced acidosis. Inhibition of Na+/H+ ex-
change by amiloride during basolateral Cl- removal resulted in
a reversible decrease in pHj, confirming that Na+/H+ exchange
is still active in the absence of basolateral Cl-. This suggests
that Na+/H+ exchange activity occurs, but that the maximal
rate of H+ extrusion is inadequate to return pHj to normal
levels and that the luminal Cl-/HCO3 exchanger has a large
capacity for extruding HCO3, at least under certain condi-
tions.

In summary, these studies provide the first direct examina-
tion of the pHj regulatory mechanisms of the various cell types
of the heterogeneous rabbit CCT. Principal cells are shown to
have both a basolateral Na+/H+ exchanger and a previously
unidentified basolateral Cl-/HCO3 exchanger. The principal
cell Cl-/HCO3 exchanger is relatively inactive at baseline pHj.
Studies of single intercalated cells utilized basolateral Cl-
removal to functionally separate α- and β-intercalated cells and
allow their individual study. The α-intercalated cell is func-
tionally identified by intracellular alkalosis in response to
peritubular Cl- removal; the β-intercalated cell by intracellular
acidosis. In addition to direct functional evidence of β-inter-
calated cell luminal Cl-/HCO3 exchange, a basolateral Na+/H+
exchanger in the β-intercalated is demonstrated. Thus, both of
these adjacent cell types have a basolateral Na+/H+ exchanger.
all three cell types of the CCT, but the polarity of distribution and basal activity differs, suggesting different functions for the Cl⁻/HCO₃⁻ exchangers in these neighboring cells.

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References

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