Basolateral Na-H Exchange in the Rabbit Cortical Collecting Tubule

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ABSTRACT We used the intracellular absorbance spectrum of the dye 4',5'-dimethyl-5-(and-6-)carboxyfluorescein (Me2CF) to measure intracellular pH (pHi) in the isolated, perfused cortical collecting tubule (CCT) of the rabbit nephron. The incident spot of light was generally 10 μm in diameter, large enough to illuminate from two to six cells. No attempt was made to distinguish principal from intercalated cells. All experiments were carried out in HCO3-free Ringer to minimize HCO3 transport. When cells were acid-loaded by briefly exposing them to Ringer containing NH4 and then withdrawing the NH4, pHi spontaneously recovered from the acid load. The pHi recovery was best fit by the sum of two exponentials. When the acid loading was performed in the absence of Na+, the more rapid of the two phases of pHi recovery was absent. The remaining slow phase never returned pHi to normal and was sometimes absent. Returning Na+ to the lumen had only a slight effect on the pHi recovery. However, when Na+ was returned to the basolateral (i.e., blood-side) solution, pH recovered rapidly and completely. The apparent K_m for basolateral Na+ was 27.3 ± 4.5 mM. The basolateral Na-dependent pHi recovery was reversibly inhibited by amiloride. We conclude that the mechanism responsible for the rapid phase of pHi recovery is an Na-H exchanger confined primarily, if not exclusively, to the basolateral membrane of the CCT.

INTRODUCTION

The cortical collecting tubule (CCT) of the rabbit plays a major role in the transport of Na+, K+, and HCO3 by the kidney. Experiments on the isolated, perfused CCT have shown that this segment reabsorbs Na+ and secretes K+ (Grantham et al., 1970). If the donor animal is fed a normal or acid diet, the CCT reabsorbs HCO3 (McKinney and Burg, 1977, 1978a; Koeppen and Helman, 1982), whereas if the animal is fed an alkaline diet, HCO3 is secreted (McKinney and Burg, 1977, 1978b; Lombard et al., 1983). Moreover, there is evidence that transport function may be segregated by cell type. The more
numerous principal cells (Kaissling and Kriz, 1979) appear to be involved in Na\(^+\) and K\(^+\) transport (Wade et al., 1979; O'Neil and Hayhurst, 1984), whereas the intercalated cells, which are rich in carbonic anhydrase (Dobyan et al., 1982), may be responsible for HCO\(_3^-\) transport. Recent evidence on the rabbit CCT (Schwartz and Al-Awqati, 1985) and its analogue, the turtle bladder (Stetson and Steinmetz, 1985), suggests that HCO\(_3^-\) reabsorption and secretion may be mediated by different subpopulations of these carbonic anhydrase-rich cells.

There is still uncertainty about the mechanisms of H\(^+\) and HCO\(_3^-\) transport in each of these cell types. Although the principal cells are not thought to be involved in the transepithelial transport of H\(^+\) or HCO\(_3^-\), they presumably have a requirement for the regulation of their internal pH that is similar to that of other cells (see Roos and Boron, 1981). This could be accomplished, as in most vertebrate cells, by an Na-H exchanger (Boron, 1983). Considering the likely transmembrane gradients for H\(^+\) and Na\(^+\), one would predict that such an Na-H exchanger would probably be at the basolateral, rather than the luminal, membrane (Boron, 1983). By analogy to the turtle bladder (see reviews by Steinmetz and Andersen, 1982; Al-Awqati, 1978), it is thought that the cells responsible for HCO\(_3^-\) reabsorption (presumably the intercalated cells or a subpopulation thereof) possess an electrogenic H\(^+\) pump that extrudes H\(^+\) across the luminal membrane. In addition, it is thought that HCO\(_3^-\) exits the cell across the basolateral membrane via a Cl-HCO\(_3^-\) exchanger. By analogy with the turtle bladder (Cohen, 1980), the cells that secrete HCO\(_3^-\) may have a Cl-HCO\(_3^-\) exchanger at the luminal membrane, and an unspecified H\(^+\)-extruding mechanism at the basolateral membrane. The luminal Cl-HCO\(_3^-\) exchanger hypothesis is consistent with the observation that alkali secretion by the CCT is influenced by the concentration of luminal Cl\(^-\) (Laski et al., 1983). The observation that transepithelial HCO\(_3^-\) secretion is not inhibited by the removal of Na\(^+\) (Schuster, 1985; Star et al., 1985) suggests that the basolateral H\(^+\)-extruding mechanism is not an Na-H exchanger.

One approach for determining the identity and location of H\(^+\) and HCO\(_3^-\) transport systems in the CCT is to investigate the mechanisms by which intracellular pH (pH\(_i\)) is regulated. Such a study could also identify mechanisms of potential importance for the transepithelial transport of HCO\(_3^-\). In the present study, we have examined pH\(_i\) regulation in the isolated, perfused CCT. pH\(_i\) was calculated from the intracellular absorbance spectrum of the pH-sensitive dye 4',5'-dimethyl-5- (and -6-) carboxyfluorescein (Me\(_2\)CF), an approach previously tested in the salamander proximal tubule (Chaillet and Boron, 1985). Because the incident light was a spot 10 μm in diameter, focused on a group of two to six random cells, our pH\(_i\) measurements presumably reflect a weighted average of the pH\(_i\) values of both principal and intercalated cells. No attempt was made to distinguish these cell types. We found that the recovery of pH\(_i\) from an intracellular acid load usually occurred in two distinct phases. One was independent of Na\(^+\) and relatively slow. The other required basolateral Na\(^+\), was inhibited by amiloride, and was substantially faster. Inasmuch as this rapid component is present in the absence of HCO\(_3^-\), it is probably mediated by a basolateral Na-H exchanger.
A portion of this work has been published in abstract form (Chaillet and Boron, 1984).

METHODS

General

The chamber and the optical system were the same as those described in the preceding paper (Chaillet and Boron, 1985), except that the diameter of the beam of light focused on the tubule was usually 10 μm. Absorbance spectra of the intracellular dye were recorded approximately once every 2 s by computer.

**TABLE I**

**Compositions of Solutions**

| Component | (1) Standard HEPES | (2) NH₄⁺ HEPES | (3) pH 6.8 HEPES | (4) 0-Na⁺ HEPES | (5) 0-Na⁺, NH₄⁺ HEPES | (6) 0-Na⁺, high-K⁺ HEPES |
|-----------|--------------------|---------------|-----------------|-----------------|------------------------|------------------------|
| Na⁺       | 146.4              | 126.4         | 141.6           | 0               | 0                      | 0                      |
| K⁺        | 5.0                | 5.0           | 5.0             | 5.0             | 5.0                    | 105.0                  |
| NH₄⁺      | 0                  | 0             | 0               | 0               | 0                      | 0                      |
| NMDG⁺     | 0                  | 0             | 0               | 0               | 0                      | 0                      |
| Mg²⁺      | 1.2                | 1.2           | 1.2             | 1.2             | 1.2                    | 1.2                    |
| Ca²⁺      | 1.0                | 1.0           | 1.0             | 1.0             | 1.0                    | 1.0                    |
| meq(+)    | 155.8              | 155.8         | 151.0           | 155.8           | 155.8                  | 155.8                  |

| Component | (1) Standard HEPES | (2) NH₄⁺ HEPES | (3) pH 6.8 HEPES | (4) 0-Na⁺ HEPES | (5) 0-Na⁺, NH₄⁺ HEPES | (6) 0-Na⁺, high-K⁺ HEPES |
|-----------|--------------------|---------------|-----------------|-----------------|------------------------|------------------------|
| Cl⁻       | 122.0              | 122.0         | 128.0           | 122.0           | 122.0                  | 122.0                  |
| H₂PO₄⁻    | 0.4                | 0.4           | 1.0             | 0.4             | 0.4                    | 0.4                    |
| HPO₄²⁻    | 1.6                | 1.6           | 1.0             | 1.6             | 1.6                    | 1.6                    |
| Acetate⁻  | 10.0               | 10.0          | 10.0            | 10.0            | 10.0                   | 10.0                   |
| HEPES⁺    | 17.8               | 17.8          | 7.6             | 17.8            | 17.8                   | 17.8                   |
| SO₄²⁻     | 1.2                | 1.2           | 1.2             | 1.2             | 1.2                    | 1.2                    |
| meq(−)    | 155.8              | 155.8         | 151.0           | 155.8           | 155.8                  | 155.8                  |

| Component | (1) Standard HEPES | (2) NH₄⁺ HEPES | (3) pH 6.8 HEPES | (4) 0-Na⁺ HEPES | (5) 0-Na⁺, NH₄⁺ HEPES | (6) 0-Na⁺, high-K⁺ HEPES |
|-----------|--------------------|---------------|-----------------|-----------------|------------------------|------------------------|
| Glucose   | 5.5                | 5.5           | 5.5             | 5.5             | 5.5                    | 5.5                    |
| Alanine   | 5.0                | 5.0           | 5.0             | 5.0             | 5.0                    | 5.0                    |
| HEPES     | 14.4               | 14.4          | 24.6            | 14.4            | 14.4                   | 14.4                   |
| pH        | 7.4                | 7.4           | 6.8             | 7.4             | 7.4                    | 7.4                    |

The concentrations of all components are in millimolar.

**Biological Preparation**

CCTs were obtained from pathogen-free, female New Zealand white rabbits (Dutchland, Inc., Reston, VA), weighing 2–5 lbs. Single tubules were isolated in cold HEPES Ringer (solution 1, below), titrated to pH 7.40 at 4°C. After transfer to the chamber, the tubule was perfused at 37°C by the method of Burg et al. (1966). The length of the exposed tubule between the perfusion pipettes was ~200 μm. The tubules were acclimatized at 37°C for ~1 h, part of which time was used for loading the cells with dye.

**Solutions**

The compositions of the Ringer solutions are given in Table I. The solutions were buffered with HEPES to the appropriate pH. For the determination of the intracellular dye calibration spectra, nigericin (Calbiochem-Behring Corp., La Jolla, CA) was added to
solution 6 from a stock solution (10 mM in ethanol) to a final nigericin concentration of 10 μM. The colorless dye precursor 4',5'-dimethyl-5- (and-6-) carboxyfluorescein diacetate (Me₂CFAc₂) was obtained from Molecular Probes, Inc., Junction City, OR. For loading cells with dye, Me₂CFAc₂ was added to solution 3 from a stock solution (100 mM in dimethyl sulfoxide) to a final concentration of 100 μM. N-Methyl-D-glucamine (NMDG) and HEPES were obtained from Sigma Chemical Co., St. Louis, MO. Ringer solutions with an Na⁺ concentration between 0 and 145 mM were made by mixing solutions 1 and 4. Amiloride hydrochloride (a gift of Merck, Sharpe & Dohme, West Point, PA) was sometimes added to solutions 1 and 4 and those containing intermediate concentrations of Na⁺.

RESULTS

Intracellular pH Calibration of the Dye

In the preceding paper (Chaillet and Boron, 1985), on the salamander proximal tubule, we demonstrated that steady state values of pHi calculated from absorbance spectra Me₂CF are within ±0.1 pH unit of those obtained with pH-sensitive microelectrodes. The two techniques also agree closely during rapid pHi transients. Because the absorbance spectrum of the intracellular dye in these salamander experiments differed from that obtained in vitro, we performed an intracellular dye calibration. pHi was nominally clamped to extracellular pH (pHe) by exposing the tubule to a solution containing the K-H exchanger nigericin, and having a K⁺ activity chosen to match the intracellular K⁺ activity. Intracellular dye absorbance spectra were then obtained over a range of pHi values. In the present experiments, we performed a similar intracellular dye calibration on the rabbit CCT. Inasmuch as there are no published data on the K⁺ activity of the rabbit CCT, we assumed a value of 80 mM (a concentration of -105 mM, assuming an intracellular K⁺ activity coefficient of 0.75). This represents the same fraction of total osmolality as measured in the salamander proximal tubule (Sackin and Boulpaep, 1981), and is within the range of measured K⁺ levels in mammalian tubules in general (see Boron and Sackin, 1983). Furthermore, we have shown that in the salamander (Chaillet and Boron, 1985), modest changes in [K⁺]i have little effect on the intracellular calibration spectra.

Fig. 1A illustrates an experiment on the CCT in which we varied pHe, from 5.89 to 8.15 in the presence of 10 μM nigericin and 105 mM K⁺ (solution 6). The ordinate is the ratio of the peak absorbance (510 nm) to the absorbance at the in vitro isosbestic wavelength (470 nm). Representative spectra, obtained after A(510)/A(470) reached a steady value, are plotted in Fig. 1B, scaled to an absorbance of unity at 470 nm. The A(510)/A(470) ratios from the spectra of Fig. 1B are plotted as a function of pH in Fig. 1C. The curve drawn through the points is the result of a nonlinear least-squares fit to a standard pH titration curve, having a pKₐ of 7.36 ± 0.01 at 37°C. In the salamander proximal tubule, the in vivo pKₐ at 25°C is 7.35 ± 0.03, shifted to a value 0.44 greater than the in vitro pKₐ (Chaillet and Boron, 1985). Although we have no in vitro data at 37°C, it is likely that a similar pKₐ shift occurs in the rabbit CCT.
The above calibration procedure is subject to the same uncertainties as outlined in the preceding work on salamander proximal tubules (Chaillet and Boron, 1985). Because these uncertainties are of a quantitative nature, however, they are unlikely to affect the general conclusions of the present study.

The measured value of pH, in these experiments is necessarily a mean value for all the cells illuminated by the incident light (see Discussion). Our spot of

![Graphical representation of the calibration procedure](image_url)

**Figure 1.** Calibration of intracellular dye. (A) Time course of $A(510)/A(470)$ while pH was varied. See text for details. The initial $A(510)/A(470)$ at pH 8.15 (solution 6) was 2.53. (B) Absorbance spectra obtained during periods of stable $A(510)/A(470)$ in the experiment of A. (C) In vivo dye calibration curve. The points are derived from the spectra of B. The curve drawn through the points is a nonlinear least-squares fit to the data. The pKₐ is 7.36 ± 0.01, and the upper and lower asymptotes are 2.84 ± 0.01 and 0.78 ± 0.01, respectively.
light was sufficiently small (i.e., 10 μm) that, in some experiments, probably only cells of the majority type (i.e., principal cells) were illuminated. Only in this fortuitous case would the measured pH$_i$ pertain to a single cell type. In most experiments, cells of the minority type (i.e., intercalated cells) were probably also illuminated, so that the measured pH$_i$ would pertain to both cell types.

**FIGURE 2.** pH$_i$ recovery from an NH$_4^+$-induced acid load in the presence and absence of Na$^+$. The pH$_i$ recovery (de) from the first NH$_4^+$-induced acid load had a rapid and a slower phase. The time constants of this double-exponential pH$_i$ recovery were 16.3 ± 1.4 and 187.3 ± 10.4 s, respectively. In the absence of Na$^+$, the pH$_i$ recovery (hi) from the acid load lacked the rapid phase. The remaining slow phase had a time constant of 231.0 ± 16.8 s. At the end of the experiment, intracellular dye calibration spectra were obtained. The resultant in vivo pH titration curve was then used to compute the ordinate scale.

In a total of 38 CCTs, the average initial absorbance at 470 nm was 0.113 ± 0.006. Assuming an average total path length of 15 μm through the tubule cells, and an extinction coefficient of 1.9 × 10$^4$ M$^{-1}$ cm$^{-1}$, this corresponds to an average intracellular dye concentration of 4.0 ± 0.2 mM. This is within the range of 2–4 mM for the intracellular dye concentration in the previous study on the salamander proximal tubule.

*Recovery of pH$_i$ from an Acid Load*

Fig. 2 illustrates an experiment in which cells of a CCT were twice acid-loaded by a 2-min exposure to 20 mM NH$_4^+$ (Boron and De Weer, 1976). The rapid rise
of pHᵢ during ab was due to the influx of the weak-base NH₃. The subsequent slow decline of pHᵢ (bc) was caused by the slower, passive influx of the weak-acid NH₄⁺, possibly augmented by Cl-OH exchange or by NH₄⁺ uptake mediated by the Na pump. Upon removal of the external NH₄⁺, pHᵢ rapidly fell (cd) to a value ~0.7 below the initial value (compare a and d). In the presence of extracellular Na⁺, pHᵢ spontaneously recovered (de) from this acid load, as a result of one or more pHᵢ-regulatory mechanisms. The time course of this pHᵢ recovery is fitted by the sum of two exponentials, a rapid phase with a time constant of 16.3 ± 1.4 s and a slow phase with a time constant of 187.3 ± 10.4 s. The tubule cells were then acid-loaded for a second time (e–h), but in the total absence of extracellular Na⁺ (replaced by NMDG⁺, solutions 5 and 4). This Na⁺ removal blocked the rapid-phase pHᵢ recovery, leaving only a slow phase (hi) with a time constant of 231.0 ± 16.8 s. The similarity in slow-phase time constants for segments de and hi suggests, but does not prove, that the slow phases in the presence (de) and absence (hi) of Na⁺ may be mediated by the same Na-independent mechanism.

We performed a total of nine experiments in which the pHᵢ recovery in the absence of Na⁺ was allowed to continue until pHᵢ reached a stable value. Fig. 3A illustrates one of three experiments in which the slow-phase pHᵢ recovery was virtually absent. Fig. 3B is one example of the remaining six experiments, in which the slow-phase pHᵢ recovery was present. The measured pHᵢ clearly stabilized at a level far below the initial value. In these six experiments, the average pHᵢ recovery amounted to 14.6% of the initial acid load.

**Calculation of Intracellular Buffering Power**

Experiments of the type shown in Fig. 3 can also be used to determine intracellular buffering power (see Boron, 1977). By definition, the intracellular buffering power is the magnitude of the intracellular acid load (i.e., the amount of strong acid added to the cell, given in millimolar) divided by the resultant fall of pHᵢ. The magnitude of the acid load is the calculated [NH₄⁺] at the time just before removal of external NH₄⁺. Upon removal of the external NH₄⁺ and NH₃, this internal NH₄⁺ dissociates into NH₃ (which passively leaves the cell) and H⁺. The latter is trapped inside and constitutes the intracellular acid load. The pHᵢ decrease is simply the fall of pHᵢ that occurs when the external NH₄⁺ is removed. The accuracy with which we can measure the pHᵢ decrease is limited by two factors, both of which relate to the low values to which pHᵢ falls after removal of the external NH₄⁺. First, such low pHᵢ values are far from the apparent pK of the dye, limiting the dye’s sensitivity. Second, the effects of nigericin calibration errors can be expected to be greatest at extreme pHᵢ values (Chaillet and Boron, 1985).

An accurate determination of intracellular buffering power also requires the blockade of all pH-related ion transport systems. This requirement was approximately met in a total of 14 experiments, the 9 discussed in conjunction with Fig. 3, and 5 others. In all cases, the Na-H exchanger was blocked by Na⁺ removal, and potential HCO₃ transporters were inhibited by the nominal removal of HCO₃⁻. Although the slow-phase mechanism of Fig. 3B was not blocked, the resultant pHᵢ changes were so slow as to negligibly affect the calculation. The result was a mean intracellular buffering power of 24.7 ± 1.7 mM. This value
includes the buffering power of all non-CO$_2$ or intrinsic intracellular buffers, and could be contaminated to some extent by HEPES buffer, which may have entered the cells.

**FIGURE 3.** Two examples of pH$_i$ recovery in the absence of Na$^+$. In both A and B, the cells were acid-loaded with a brief pulse of 20 mM NH$_4^+$ to the lumen and bath in the absence of Na$^+$ (solution 5). Removal of NH$_4^+$, in the continued absence of Na$^+$ (solution 4), caused a rapid acidification in both examples. In A, there was no recovery of pH$_i$. In B, pH$_i$ partially recovered, with a time constant of 124 s.
The Na⁺ Dependence of the Rapid-Phase Mechanism

Fig. 2 demonstrated that the rapid-phase pHᵢ recovery mechanism is blocked by removing Na⁺ from both the lumen and bath. To determine the sidedness of this Na⁺ dependence, we performed the experiment of Fig. 4. In the absence of Na⁺, the pHᵢ recovery (ab) from the NH₄⁺-induced acid load was very slow. This suggests that the slow-phase mechanism is relatively inactive. The addition of 145 mM Na⁺ to only the lumen produced a very slow pHᵢ recovery (bc). The subsequent removal of luminal Na⁺ caused a slow pHᵢ decline (cd). When 145 mM Na⁺ was then added to the bath alone, pHᵢ recovered rapidly and completely (de). The maximal rate of pHᵢ recovery in de (0.0182 pH/s) was 13-fold greater than that in bc (0.0014 pH/s). In a total of five similar experiments, the factor was 13.7 ± 4.7. Thus, the Na-dependent, rapid-phase mechanism is confined primarily, if not exclusively, to the basolateral membrane.

In order to quantify the basolateral Na⁺ dependence of the rapid-phase pHᵢ recovery, we performed a series of experiments similar to the one of Fig. 5. After the cells were acid-loaded in the absence of luminal and basolateral Na⁺, the slow-phase mechanism caused a partial pHᵢ recovery (ab), followed by a slow and modest decline of pHᵢ to a stable value (bc). Then, in the absence of any slow-phase contribution to pHᵢ recovery, we determined the effect of three concentrations of basolateral Na⁺ on the rate of rapid-phase pHᵢ recovery. When 4.4 mM Na⁺ was added, the pHᵢ recovery was approximately linear, with a rate
of 0.0011 pH/s (cd). This Na-induced recovery was halted upon removal of basolateral Na⁺, and pHᵢ slowly declined (de). The addition of 29 mM Na⁺ (ef) resulted in a substantially higher initial rate of pHᵢ recovery, 0.0063 pH/s. The subsequent removal of Na⁺ caused a pHᵢ decline (fg). This acidification is consistent with a persistent acid load in the absence of the Na-dependent acid extrusion mechanism or, more likely, a reversal of the Na-dependent mechanism. Indeed, we observed that amiloride, an inhibitor of Na-H exchange, slowed this pHᵢ decline (not shown). Finally, the addition of 145 mM Na⁺ (solution 1) resulted in a rapid, complete recovery of pHᵢ (gh). Segment gh was well fitted by a single exponential, having a time constant of 41.2 s.

Because the rates of pHᵢ recovery are clearly pHᵢ dependent (note the exponential shape of the pHᵢ recovery in segment gh in Fig. 5), we compared pHᵢ recovery rates for the three Na⁺ concentrations at the same pHᵢ. The aforementioned pHᵢ recovery rates in 4.4 and 29 mM Na⁺ were obtained at the same pHᵢ, ~6.85. In order to estimate the recovery rate in 145 mM Na⁺ at this same pHᵢ, we fitted the gh pHᵢ recovery to a single exponential and extrapolated this fitted exponential curve down to pH 6.85. At this pHᵢ, the extrapolated curve had a slope of 0.0163 pH/s. We performed a series of similar experiments at basolateral Na⁺ concentrations of 4.4, 15, 29, and 145 mM Na⁺. In each case, the pHᵢ recovery rates at these concentrations were normalized to the rate at 145 mM
Na⁺, at the same pHᵢ. This analysis assumes that [Na⁺]ᵢ is the same at different basolateral Na⁺ concentrations. Because the cells were exposed to Na-free solutions for such a long time before the addition of basolateral Na⁺, it is likely that [Na⁺]ᵢ was uniformly low in all cases. However, the kinetics at a low [Na⁺]ᵢ are not necessarily the same as at a more physiological value. These data are presented in Fig. 6. The curve is the result of a nonlinear least-squares curve fit to the Michaelis-Menten equation. The apparent $K_m$ for basolateral Na⁺ was 27.3 ± 4.5 mM. These results are consistent with the hypothesis that the rapid-phase pHᵢ recovery is caused by a basolateral Na-H exchanger.

**Figure 6.** Basolateral Na⁺ dependence of the rapid-phase pHᵢ recovery. The data are derived from the experiment of Figs. 5 and 16 similar experiments. For each experiment, the initial pHᵢ recovery rates in 4.4 (n = 6), 15 (n = 10), or 29 (n = 7) mM Na⁺ were normalized to the recovery rate at the same pHᵢ in 145 mM Na⁺. The mean initial recovery rate in 145 mM Na⁺ was 0.014 pH/s. The normalized recovery rates are plotted vs. [Na⁺]ₒ, with the vertical lines indicating standard errors. The curve drawn through the points is the result of a nonlinear least-squares fit to a variant of the Michaelis-Menten equation (Boron, 1985) that forces the curve to pass through the mean initial recovery rate in 145 mM Na⁺ (indicated by the asterisk). $V_{max}$ was 0.0167 ± 0.0028 pH/s, and $K_m$ was 27.2 ± 4.5 mM.

**Amiloride Sensitivity of the Rapid-Phase pHᵢ Recovery Mechanism**

In order to further test the hypothesis that the rapid-phase pHᵢ recovery is due to a basolateral Na-H exchanger, we examined the sensitivity of the rapid-phase mechanism to amiloride, a competitive inhibitor of Na-H exchange (Kinsella and Aronson, 1981). Fig. 7A illustrates an experiment in which cells were acid-loaded in the absence of Na⁺. When the NH₄⁺ was removed, 50 μM amiloride was simultaneously added to the bath. In the absence of Na⁺, pHᵢ did not recover (ab). Upon the addition of 15 mM Na⁺ to the bath, the recovery of pHᵢ was approximately linear, with a rate of 0.0015 pH/s (bc). The subsequent simulta-
Figure 7. Effect of basolateral amiloride on the rapid-phase $pH_i$ recovery. (A) After a brief exposure to 20 mM NH$_4^+$ in the absence of Na$^+$ (solution 5), 50 µM amiloride was added to the bath at the same time that NH$_4^+$ was removed (solution 4). The addition of 15 mM Na$^+$ to the bath, in the continued presence of 50 µM amiloride, caused pH$_i$ to recover at a rate of 0.0015 pH/s (bc). The addition of 15 mM Na$^+$ in the absence of amiloride caused pH$_i$ to recover with an initial rate of 0.0065 pH/s (de). (B) In an experiment similar to that of A, but on a different tubule, the effect of 1 mM amiloride was tested. The addition of 15 mM Na$^+$ to the bath in the presence of amiloride caused only a negligible pH$_i$ recovery (hc), whereas the addition of 15 mM Na$^+$ to the bath in the absence of amiloride caused pH$_i$ to recover with an initial rate of 0.0061 pH/s.
neous removal of Na\(^+\) and amiloride caused pH\(_i\) to slowly decline and level off \((cd)\). The addition of 15 mM bath Na\(^+\), now in the absence of amiloride, caused pH\(_i\) to recover more rapidly \((de)\), with an initial rate of 0.0065 pH/s. The difference in the pH\(_i\) recovery rates between \(bc\) and \(de\) corresponds to an inhibition by amiloride of 77\%. In a total of three similar experiments, 50 \(\mu\)M amiloride inhibited the pH\(_i\) recovery in 15 mM Na\(^+\) by 62 ± 7\%. The experiment of Fig. 7B is similar to that of Fig. 7A, except that 1 mM amiloride was present in the bath. Whereas in Fig. 7A there was still a partial pH\(_i\) recovery in the presence of 50 \(\mu\)M amiloride, 1 mM amiloride completely inhibited pH\(_i\) recovery in 15 mM Na\(^+\) (compare \(bc\) and \(de\) in Fig. 7B). Taken together, the Na\(^+\) dependence and the amiloride sensitivity indicate that the rapid-phase mechanism is a basolateral Na-H exchanger.

**DISCUSSION**

Relative Contributions of Principal vs. Intercalated Cells to Measured pH\(_i\) Values

Inasmuch as the CCT has been shown to be composed of about two-thirds principal cells and one-third intercalated cells (Kaissling and Kriz, 1979), in most cases the pH\(_i\) values we measured for the CCT reflect an average pH\(_i\) for both cell types. Their relative contributions to the measured pH\(_i\) depend in a complex way on at least five parameters, each of which may have different values in the two cell types.

(a) pH\(_i\) determines not only the shape of the absorbance spectrum, but its sensitivity to pH changes. Thus, all else being equal, cells with pH\(_i\) values nearer the pK of the dye tend to make greater contributions to the mean pH\(_i\).

(b) The position of the cells in the light path also influences the degree to which they contribute to the average pH\(_i\). For cells in series with one another, it is easily shown that the absorbances are additive, and that the average pH\(_i\) is weighted in favor of the cell with the higher dye absorbance (which is affected both by pH\(_i\) and dye concentration). For cells in parallel with one another, it can be shown that the average absorbance is a weighted mean\(^1\) of the individual absorbances, and that the average pH\(_i\) is weighted in favor of the cell with the lower dye absorbance.

(c) The spectral properties of Me\(_2\)CF in vitro are different from those of Me\(_2\)CF incorporated into salamander proximal tubule cells (Chaillet and Boron, 1985). In particular, the intracellular dye has a relatively flattened absorbance spectrum that is red-shifted by ~5 nm, as well as an elevated pK\(_a\). Should small differences exist between the spectral properties of the dye in different cell types of the rabbit CCT, the average pH\(_i\) could be biased toward one of them.

(d) The projected area and thickness that the cells present to the light path also affect the contribution of their pH\(_i\) values to the average pH\(_i\). Regardless of

\(^1\) For \(n\) cells that are parallel with one another in the light path, each with an absorbance \(A_n\), and each illuminated by a fraction \(f_i\) of the total incident light, the average absorbance \(A\) is given by:

\[A = -\log \sum_{i=1}^{n} [f_i \cdot 10^{-A_i}]\]
whether the cells are in parallel or in series, other factors being equal, the average pH is weighted toward the cell for which the projected area in the light path is greater. If cells have different thicknesses, however, the average pH will be biased toward the cell with the greater thickness if the cells are in series, and toward the cell with the lesser thickness if the cells are in parallel (see b above).

(e) The effect of dye concentration on average pH can be predicted from b above. For cells in series, all other things being equal, the average pH is weighted toward the cell having the greater dye concentration, whereas for cells in parallel, the opposite is true. There are no data on the relative intracellular concentrations of fluorescein derivatives for the cell types of the CCT. However, one would expect [Me2CF] to depend on at least three factors: (i) the rate at which the dye precursor (an ester derivative of the dye) enters the cell during the period of dye loading, (ii) the rate at which the intracellular dye is formed as the precursor is hydrolyzed spontaneously and by native intracellular esterases, and (iii) the rate at which the dye leaks from the cell. In their experiments on Ehrlich ascites tumor cells, J. A. Thomas et al. (1979) found that the appearance of intracellular fluorescein derivatives depends critically upon extracellular pH in a way that suggests that it is the protonated, neutral form of the esterified dye precursor that enters the cell. We have noted a similar trend in our experiments. Regarding the rate of hydrolysis of Me2CFAc2 by intracellular esterases, it is interesting to note that carbonic anhydrase II, which is present at high levels in the intercalated cells of the CCT, has weak esterase activity (Hopkinson et al., 1974). However, there are no data that address the issue of whether carbonic anhydrase II significantly increases the esterase activity of intercalated cells, or whether there is a difference in relative esterase activities of principal and intercalated cells. Finally, as far as factor iii is concerned, we have noted that one of the most important determinants of [Me2CF] in our experiments is the rate at which the dye leaks from the cells. It should be noted that experiments can be divided into two parts, the period of dye loading, and the period during which pH is actually measured. During the loading period, [Me2CF], is determined by the interaction of factors i-iii. The subsequent time course of [Me2CF], depends on the [Me2CF], obtaining at the end of the loading period, and on the dye leakage rate. The actual importance of factors i-iii for determining [Me2CF], in either the CCT as a whole or in the individual cell types is not known.

Even though the measured pH is a complex average of the pH values of the different cell types of the CCT, changes in this measured pH can still provide valuable information about individual acid/base transport systems. This is exemplified by the experiment of Fig. 5, in which kinetic details of Na-H exchange could be assessed even though the CCT possesses a second mechanism for pH recovery.

The Two Phases of pH Recovery from an Intracellular Acid Load

A major conclusion of this study is that there are at least two mechanisms by which the pH, of the CCT can increase after an acute intracellular acid load. The first, or slow-phase, mechanism is independent of Na*, and causes only a partial recovery of the apparent pH. Furthermore, the slow-phase mechanism
was virtually absent in about one-third of the experiments in which we carefully
searched for it. This slow-phase pH recovery could simply have been due to the
passive transport of H⁺ or a buffer such as HEPES, or to the electrogenic H⁺
pump that is believed to be present in this nephron segment. Inasmuch as there
are no specific inhibitors of the H⁺ pump, and the nonspecific inhibitors would
be expected to cause pH changes for other reasons, we made no attempt to
further characterize the slow-phase pH recovery mechanism. We conclude that
the second, or rapid-phase, mechanism for pH recovery from an acid load is
probably a basolateral Na-H exchanger (see below).

It is possible that the slow-phase and/or the rapid-phase mechanisms are present
in both principal and intercalated cells. This hypothesis requires that the slow-
phase mechanism be absent in about one-third of the experiments, and that it
not be capable of accomplishing a complete pH recovery in the absence of Na⁺.
An alternative hypothesis is that the two phases of pH recovery have their origins
in the two cell types, the intercalated cells (or a subpopulation thereof) and the
principal cells. The intercalated cell, which is analogous to the "dark" or "mito-
chondria-rich" cell of the turtle bladder, constitutes about one-third of the total
cell number in the CCT (Kaissling and Kriz, 1979), and is believed to possess an
H⁺ pump. Our data obtained in Na-free Ringer also can be accounted for if (a)
the slow-phase mechanism is confined to the intercalated cells (or a subpopula-
tion), and (b) the rapid-phase mechanism is present in at least the principal cells,
and possibly in the intercalated cells as well. In experiments in which the
illuminating spot of light fell only on principal cells, the measured pH would not
recover at all from an acid load, inasmuch as the Na-H exchanger would be
blocked by Na⁺ removal. In experiments in which the spot fell on both interca-
lated and principal cells, the apparent pH would only partially recover. That is,
the pH of the minority, intercalated cells might recover fully or partially, whereas
the pH of the majority, principal cells would once again fail to recover. As a
result, the mean pH of the illuminated cells would only partially recover. This
hypothesis can be tested only by studying pH transients in identified intercalated
and principal cells.

Characteristics of the Basolateral Na-H Exchanger
A second conclusion of this study is that the rapid-phase mechanism of pH recovery is due to a basolateral Na-H exchanger. Although other Na-dependent,
P⁺-regulating mechanisms have been described (R. C. Thomas, 1977; Boron et
al., 1981; Boron and Russell, 1983; Boron and Boulaep, 1983b), these are all
HCO₃⁻ dependent. Inasmuch as our experiments were performed in the nominal
absence of HCO₃⁻, it is unlikely that the rapid-phase pH recovery mechanism
could be mediated by one of these aforementioned HCO₃⁻ transporters. Furth-
more, the amiloride sensitivity of this mechanism strongly suggests that this Na-
dependent mechanism is an Na-H exchanger. Our apparent Kₘ for external Na⁺,
27 mM, is somewhat larger than the values of 6.3 (Kinsella and Aronson, 1981)
and 13.5 mM (Warnock et al., 1982) obtained for Na-H exchangers of brush-
border membrane vesicles of the rabbit renal cortex. However, our value is
somewhat lower than the value of 42 mM (at pHₐ 7.5) for the Na-H exchanger.
of the MDCK kidney cell line (Rindler and Saier, 1981). Our observed inhibition of the Na-H exchanger with 50 \( \mu \text{M} \) amiloride in the presence of 15 mM Na\(^+\) (i.e., 62\%) is also consistent with the apparent inhibition constants found in membrane vesicles (Kinsella and Aronson, 1981). The Na-H exchanger described in the present study is stimulated by intracellular acid loads (i.e., low values of pH). If we assume the intracellular buffering power to be reasonably independent of pH\(_i\), then the exponential time course of the pH\(_i\) recovery implies that the Na-H exchange rate is high at low pH\(_i\) values, and falls linearly toward zero as pH\(_i\) approaches a "threshold" value of \( \sim 7.5 \). This pH\(_i\) dependence is shared with the HCO\(_3^-\)-dependent pH\(_i\) regulator of invertebrates as well as the Na-H exchanger of vertebrate cells (see Roos and Boron, 1981; Boron, 1983). The only other report of an Na-H exchanger in a native renal cell concerns the salamander proximal tubule, which was studied with pH\(_i\) and Na-sensitive microelectrodes (Boron and Boulpaep, 1983a). This preparation is noteworthy in that it possesses both luminal and basolateral Na-H exchangers.

**Significance of the Basolateral Na-H Exchanger**

**Possible Role in Transepithelial HCO\(_3^-\) Secretion** Our data suggest that most, and possibly all, cells of the CCT possess a basolateral Na-H exchanger. Such an exchanger could participate in two activities: alkali secretion and pH\(_i\) regulation. The luminal step of alkali secretion would presumably involve the efflux of HCO\(_3^-\), possibly in exchange for Cl\(^-\), and would acidify the cell interior. If an Na-H exchanger were present in the basolateral membrane of the same cell, this intracellular acid load would stimulate the Na-H exchanger to extrude acid across the basolateral membrane, and thereby complete the process of transepithelial HCO\(_3^-\) secretion. However, the recent observations by Schuster (1985) and Star et al. (1985), that Na\(^+\) is not required for HCO\(_3^-\) secretion, indicate that Na-H exchange does not play an important role in this process. This suggests that the cells responsible for HCO\(_3^-\) secretion have at most a small degree of basolateral Na-H exchange activity.

**Role in pH\(_i\) Regulation** All cells of the CCT must deal with the problem of pH\(_i\) regulation. The intercalated cells, for which there may be two subpopulations (Schwartz and Al-Awqati, 1985; Stetson and Steinmetz, 1985), could in principle accomplish this pH\(_i\) regulation by means of the same H\(^+\) pump that presumably is responsible for HCO\(_3^-\) reabsorption and secretion. Our data are consistent with this hypothesis, as well as the variant in which intercalated cell pH\(_i\) regulation is augmented by a basolateral Na-H exchanger. We propose that pH\(_i\) regulation for the principal cells is probably accomplished by the basolateral Na-H exchanger identified in the present study. This hypothesis is supported by the fact that we observed the basolateral Na-H exchanger in all our experiments. The confinement of the Na-H exchanger to the basolateral membrane might be expected (Boron, 1983), inasmuch as the in vivo lumen-to-cell gradients for Na\(^+\) and H\(^+\) could be unfavorable for acid extrusion by Na-H exchange. Similar basolateral Na-H exchangers might also be anticipated in other renal tubule cells not involved in acid secretion.
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