Cellular NH$_4^+$/$K^+$ Transport Pathways in Mouse Medullary Thick Limb of Henle

Regulation by Intracellular pH

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Abstract Fluorescence and electrophysiological methods were used to determine the effects of intracellular pH (pHi) on cellular NH$_4^+$/$K^+$ transport pathways in the renal medullary thick ascending limb of Henle (MTAL) from CD1 mice. Studies were performed in suspensions of MTAL tubules (S-MTAL) and in isolated, perfused MTAL segments (IP-MTAL). Steady-state pH$_i$, measured using 2,7-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF) averaged 7.42 ± 0.02 (mean ± SE) in S-MTAL and 7.26 ± 0.04 in IP-MTAL. The intrinsic cellular buffering power of MTAL cells was 29.7 ± 2.4 mM/pHi unit at pH$_i$ values between 7.0 and 7.6, but below a pH$_i$ of 7.0 the intrinsic buffering power increased linearly to ~50 mM/pHi unit at pH$_i$ 6.5. In IP-MTAL, NH$_i$ entered cells across apical membranes via both Ba$^{2+}$-sensitive pathway and furosemide-sensitive Na$^+$:$K^+$:$\text{NH}_4^+$:$2\text{Cl}^-$ cotransport mechanisms. The $K_{a5}$ and maximal rate for combined apical entry were 0.5 mM and 83.3 mM/min, respectively. The apical Ba$^{2+}$-sensitive cell conductance in IP-MTAL ($G_c$), which reflects the apical $K^+$ conductance, was sensitive to pH$_i$ over a pH$_i$ range of 6.0-7.4 with an apparent $K_{a5}$ at pH$_i$ ~6.7. The rate of cellular NH$_i^+$ influx in IP-MTAL due to the apical Ba$^{2+}$-sensitive NH$_i^+$ transport pathway was sensitive to reduction in cytosolic pH whether pH$_i$ was changed by acidifying the basolateral medium or by inhibition of the apical Na$^+$:$H^+$ exchanger with amiloride at a constant pH$_o$ of 7.4. The pH$_i$ sensitivities of $G_c$ and apical, Ba$^{2+}$-sensitive NH$_i^+$ influx in IP-MTAL were virtually identical. The pH$_i$ sensitivity of the Ba$^{2+}$-sensitive NH$_i^+$ influx in S-MTAL when exposed to (apical + basolateral) NH$_4$Cl was greater than that observed in IP-MTAL where NH$_4$Cl was added only to apical membranes, suggesting an additional effect of intracellular NH$_i^+$ on NH$_i^+$ influx. NH$_i^+$ entry via apical Na$^+$:$K^+$:$\text{NH}_4^+$:$2\text{Cl}^-$ cotransport in IP-MTAL was somewhat more sensitive to reductions in pH$_i$ than the Ba$^{2+}$-sensitive NH$_i^+$ influx pathway; NH$_i^+$ entry decreased by 52.9 ± 13.4% on reducing pH$_i$ from 7.31 ± 0.17 to 6.82 ± 0.14. These results suggest that pH$_i$ may provide a negative feedback signal for regulating
the rate of apical NH$_4^+$ entry, and hence transcellular NH$_4^+$ transport, in the MTAL.
A model incorporating these results is proposed which illustrates the role of both 
pH$_t$ and basolateral/intracellular NH$_4^+$/NH$_3$ in regulating the rate of transcellular N
H$_4^+$ transport in the MTAL.

**INTRODUCTION**

The regulated excretion of NH$_4^+$ by the kidney is required for maintenance of
systemic acid/base balance. NH$_3$ is synthesized in renal proximal tubule cells (Good
and Burg, 1984) and preferentially secreted into the lumen of this nephron segment
(Nagami and Kurokawa, 1985). Further downstream along the nephron, the thick
ascending limb of Henle (TAL) actively reabsorbs NH$_4^+$ (Good and Burg, 1984; Good,
Knepper, and Burg, 1984; Knepper, Packer, and Good, 1989). It has been proposed
that NH$_4^+$ reabsorption by the TAL plays a major role in the maintenance of a high
medullary interstitial concentration of NH$_4^+$, which in turn permits regulation of N
H$_4^+$ excretion independently of H$_2$O excretion by the terminal portion of the nephron
(Knepper et al., 1989).

Good et al. (1984) have shown that NH$_4^+$ absorption in the isolated perfused TAL
occurs in the absence of a favorable transepithelial pH gradient, indicating that
transepithelial pH trapping of NH$_3$ as NH$_4^+$ is not responsible for NH$_4^+$ reabsorption
in the TAL. We have recently described the cellular mechanisms by which the mouse
medullary segment of the TAL (MTAL) mediates active, transepithelial, pH-indepen-
dent, transcellular NH$_4^+$ transport under isotonic conditions (Kikeri, Sun, Zeidel, and
Hebert, 1989). These studies demonstrated that mouse MTAL cells are polarized
such that apical membranes are virtually impermeable to NH$_3$ but highly permeable
to NH$_4^+$, while basolateral membranes are highly permeable to NH$_3$ (Kikeri et al.,
1989). NH$_4^+$ enters mouse MTAL cells from the lumen via both an apical Ba$^{2+}$-
sensitive pathway (possibly an apical K$^+$ channel) and apical furosemide/bumetanide-
sensitive Na$^+$.K$^+$:2Cl$^-$ cotransport (Kikeri et al., 1989). NH$_4^+$ appears to be carried on
the K$^+$ site of the Na$^+$.K$^+$:2Cl$^-$ cotransporter in the TAL (Na$^+$.K$^+$.[NH$_4^+$].2Cl$^-$
cotransport [Kinne, Kinne-Saffran, Schuetz, and Schloelermann, 1986]). NH$_4^+$ exit
from mouse MTAL cells occurs by H$^+$ extrusion via apical Na$^+$.H$^+$ exchange coupled
to diffusion of NH$_3$ across basolateral membranes (Kikeri et al., 1989). The lumen-
positive transepithelial voltage in the mouse MTAL, which is due to NaCl absorption
(Hebert and Andreoli, 1984), may provide the driving force for the transport of
protons from the lumen to the basolateral (interstitial) medium via the cation-
selective paracellular pathway (Kikeri et al., 1989).

Because of the unusually high NH$_4^+$ permeability of apical membranes of MTAL
cells, exposure to either apical or apical plus basolateral NH$_4$Cl results in a large cell
acidification (Kikeri et al., 1989). Thus, NH$_4^+$ absorption by the MTAL is associated
with potentially lethal reductions in pH$_t$. Some of the possible factors that could limit
the magnitude of the NH$_4^+$-induced acidification of TAL cells include (a) the cellular
buffering power, (b) acid extrusion via Na$^+$.H$^+$ exchange (Kikeri, Azar, Sun, Zeidel,
and Hebert, 1990a) and possibly via an H$^+$-ATPase (Brown, Hirsch, and Gluck, 1988;
Kikeri et al., 1990a), and (c) feedback inhibition of NH$_4^+$ entry pathways by either the
NH$_4^+$-induced cell acidification or by intracellular NH$_4^+$/NH$_3$ itself (Oberleithner,
Munich, Schwab, and Dietl, 1986; Paris and Pouyssegur, 1986). This latter possibility
was suggested by our earlier observation that the apical NH₄⁺ entry pathways in the mouse MTAL (i.e., K⁺ channels and Na⁺:K⁺:2Cl⁻ cotransporters) did not appear to mediate significant exit of NH₄⁺ from acidified cells (Kikeri et al., 1989).

The purpose of this study was to evaluate whether some or all of the aforementioned factors contribute to modulating the effects of NH₄⁺ transport on pHₐ in the mouse MTAL. Specifically, we used both the isolated perfused MTAL tubule and suspensions of MTAL tubules/cells from CD1 mice to determine the magnitude of the intracellular buffering power of MTAL cells and to assess the effects of pHₐ on NH₄⁺ entry into MTAL cells via the Ba²⁺-sensitive NH₄⁺ transport pathway and Na⁺:K⁺(NH₄⁺):2Cl⁻ cotransporter. Intracellular pH transients due to NH₄⁺ entry into MTAL cells were measured using 2,7-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF) and rates of NH₄⁺-dependent H⁺ flux calculated using the intrinsic cellular buffer power. The results of these studies support the view that both pHₐ and basolateral/intracellular NH₄⁺/NH₃ play an important role in regulating the rates of cellular NH₄⁺ entry via the Ba²⁺-sensitive pathway and Na⁺:K⁺(NH₄⁺):2Cl⁻ cotransporter in MTAL cells. In addition, we have incorporated these results into a model that illustrates the role of both pHₐ and NH₄⁺/NH₃ in regulating NH₄⁺ absorption by the MTAL.

**METHODS**

**Cell Preparations**

Two MTAL cell preparations from CD1 mice were used: suspensions of MTAL tubules (S-MTAL) and the isolated perfused MTAL (IP-MTAL).

S-MTAL were prepared as described previously (Kikeri et al., 1990a). Briefly, the inner stripe of the outer medulla from the kidneys of three to six mice were isolated and subjected to collagenase digestion; thereafter, MTAL tubules were separated from other tubule fragments by sedimentation through 3 g/dl albumin. The vast majority of tubules in these suspensions (>97%) were (a) morphologically identical to MTAL tubules in vivo, and (b) labeled with anti-Tamm Horsfall antibody, indicating that these suspensions consisted almost purely of MTAL tubules (Kikeri et al., 1990a). MTAL tubules in suspensions were functionally intact since they exhibited high rates of ouabain-sensitive (transport-related) oxygen consumption that was inhibited by either furosemide or bumetanide, and responded to arginine vasopressin by accumulating cyclic AMP (Kikeri et al., 1990a). In addition, these tubules had open lumens, allowing access of drugs/ions/inhibitors to apical membranes (Kikeri et al., 1990a). The advantages of using S-MTAL preparations include ease of preparation and the ability to obtain a large number of paired measurements in a single preparation. We have shown previously that transport data obtained in S-MTAL preparations were virtually identical to those obtained in IP-MTAL tubules (Kikeri et al., 1989, 1990a).

In experiments using IP-MTAL, tubules were isolated from the inner stripe of the outer medulla and perfused in vitro using methods previously described in detail (Hebert, Culpepper, and Andreoli, 1981a; Hebert and Andreoli, 1984, 1986). The perfused MTAL segments were 0.2-0.3 mm in length. Use of the IP-MTAL permitted evaluation of the sidedness (i.e., polarity) of transport processes.

**Measurement of pHₐ**

Intracellular pH (pHₐ) was measured in S-MTAL or IP-MTAL with BCECF using methods described by us previously (Kikeri et al., 1989, 1990a). BCECF-loaded MTAL tubules (S-MTAL
and IP-MTAL) exhibited uniform fluorescence at both 495 and 440 nm. Background fluorescence intensities (including cellular autofluorescence) were typically <1–2% of the total fluorescence after loading with BCECF.

**S-MTAL.** Tubule suspensions were transiently exposed (15 min at 25°C) to 2.5 μM of the tetra-acetoxyethyl ester of BCECF (BCECF-AM) and then washed free of extracellular dye. Fluorescence was monitored at 37°C in a computer-controlled SLM-4000 SP-500C spectrophotometer (SLM Instruments, Inc., Urbana, IL) equipped with a water-jacketed, temperature-controlled cuvette holder and magnetic stirrer. A 50–75-μl aliquot of BCECF-loaded S-MTAL was diluted into a plastic cuvette containing 3 ml of medium and fluorescence was monitored at 530 nm emission wavelength, while the excitation wavelength rapidly alternated between 500 and 440 nm. After each experiment, the cells were pelleted in a microcentrifuge and the fluorescence of the extracellular medium was measured at both 500 and 440 nm. By subtracting the extracellular fluorescence intensities at 500- and 440-nm excitation wavelengths from the respective total fluorescence intensities obtained during the preceding experiment (due to intracellular + extracellular dye), fluorescence intensities due to intracellular dye were obtained (Kikeri et al., 1990a). We have previously shown that leakage of BCECF from S-MTAL occurs at very low rates (Kikeri et al., 1990a). The high K⁺ (110 mM)/nigericin (5 μM) method of Thomas, Buchbaum, Zimniak, and Racker (1979) was used to convert intracellular 500 nm/440 nm excitation ratios to units of pH_i over a pH_i range of 6.3–8 as described previously in detail by us (Kikeri et al., 1990a). Experiments were performed at 37°C in CO₂/HCO₃⁻-free medium containing (mM): 140 Na⁺, 5 K⁺, 148 mM Cl⁻, 1 Ca²⁺, 1 Mg²⁺, 1 PO₄⁻, 20 mannitol, 10 glucose, 10 HEPES, and 0.2 g/dl albumin, equilibrated with 100% O₂, pH 7.4.

**IP-MTAL.** Standard CO₂/HCO₃⁻-free perfusing and bathing media contained (mM): 140 Na⁺, 5 K⁺, 149.4 Cl⁻, 1 Ca²⁺, 1.2 Mg²⁺, 3 HEPES, 5 l-alanine, and 5.5 glucose, equilibrated with 100% O₂, pH 7.4, at 37°C. Bathing media also contained 0.2 g/dl Fraction V BSA. NH₄Cl, when added, replaced an equimolar amount of NaCl so that total osmolality and ionic strength remained constant. The perfusion flow rate was maintained between 10 and 20 nl/min, which is sufficient to minimize axial changes in perfusate ion concentrations and to chemically clamp the spontaneous transepithelial voltage along the length of the tubule. The flow rate of the bathing medium was maintained at 10–15 ml/min, which is sufficient to change completely the bath solution in <5 s (Hebert, Culpepper, and Andreoli, 1981b). Tubules were loaded with BCECF by transient exposure (10 min) to 10 μM BCECF-AM in the bathing medium. Fluorescence was alternately measured at excitation wavelengths of 495 and 440 nm (emission wavelength = 530 nm) using a computer-controlled inverted fluorescence microscope system (Carl Zeiss, Inc., Thornwood, NY) (Boyarsky, Ganz, Sterzel, and Boron, 1988; Kikeri et al., 1989, 1990a). Background fluorescence was subtracted from each excitation wavelength at each excitation wavelength to obtain intensities of intracellular fluorescence.

Calibration runs relating 495 nm/440 nm excitation ratio and pH_i (over a pH_i range of 6.2–7.7) were performed in three perfused tubules using medium containing 110 mM K⁺ and 5 μM nigericin (medium pH 6.2–7.7). Fluorescence ratios at the various pH_i levels in the calibration runs were normalized such that the 495 nm/440 nm ratio at a pH_i of 7.2 was arbitrarily set at a value of 1. Fig. 1 shows a computer-fitted pH titration curve relating the normalized 495 nm/440 nm excitation ratios and pH_i, as described initially by Boyarski et al. (1988). To convert fluorescence ratios obtained during an experiment on an individual IP-MTAL, the pH_i was set at 7.2 by exposure to 110 mM K⁺/5 μM nigericin (medium pH 7.2) at the end of the given experiment, and the 495 nm/440 nm ratio was then measured (single point calibration). Fluorescence ratios obtained during the experimental period were normalized such that the 495 nm/440 nm ratio obtained with the single point calibration was equal to one, and then the fitted titration curve shown in Fig. 1 was used to convert the normalized experimental ratios to units of pH_i (Boyarsky et al., 1988).
To compare initial rates of \( \frac{dpH_i}{dt} \) change in either S-MTAL or IP-MTAL, the apparent initial rate of \( pH_i \) change was obtained either by measuring the slope of a computer-fitted linear regression over the initial \( \sim 5 \) s of \( pH_i \) change, or by measuring the tangent at the initial time point of an exponential curve computer-fitted to the initial time course of \( pH_i \) change. These transformations require no assumptions as to the mechanisms of \( pH_i \) change. Correlation coefficients for these fitted curves averaged 0.96 ± 0.02.

The initial rate of acid/base flux \( J_{\text{H}} \) (millimolar per minute) at a given \( pH_i \) \( (pH_i)_x \) was calculated using measurements of \( \frac{dpH_i}{dt} \) (in \( pH_i \) units per minute) at \( (pH_i)_x \) and total buffering power \( (\beta_a \text{ millimolar per } pH_i) \) at \( (pH_i)_x \) (Fig. 3) as (Boyarsky et al., 1988):

\[
J_{\text{H}} = \left( \frac{dpH_i}{dt} \right)_{(pH_i)_x} \times (\beta_a)_{(pH_i)_x}
\]

These "flux" values in millimolar per minute can be converted to standard units (picomoles per second per square centimeter; see footnote 2) by using an MTAL tubule of 20 \( \mu \)m i.d. and a tubule cell volume of 0.25 nl/mm (Hebert, 1986). Comparisons among flux values reported in this paper and expressed in millimolar per minute assume that the surface-to-volume ratio for the tubule remains constant or changes negligibly.

**Electrical Measurements in IP-MTAL**

The electrical system used for the measurement of transepithelial voltage \( V_e \) (millivolts) and transepithelial conductance \( G_e \) (millisiemens per square centimeter) was identical to that used previously in this laboratory (Hebert et al. 1981a, b; Hebert and Andreoli, 1984, 1986). Briefly, the perfusion pipette was made from 2 mm o.d. theta-style electrode glass (Hilgenberg, Malsfeld, Germany) that was divided axially by a septum, permitting virtually complete electrical separation of perfusion and current passing circuits. The perfusion half of the pipette also served as an electrical bridge for measurement of \( V_e \) (lumen with respect to bath). Electrical connections were made to the free flowing perfusate and bath with 3 M KCl/Ag/AgCl bridges (MERE-2; World Precision Instruments, Sarasota, FL). \( V_e \) at the collecting end of the perfused tubule was measured with a Ag/AgCl wire placed into the collecting fluid and connected to a high impedance electrometer (VF-2; World Precision Instruments). Biphasic direct current pulses \( (I_o \text{ nanoamperes}) \); range = ±600; duration = 1 s) were generated by a computer-linked voltage–current clamp (VCC 600; Physiological Instruments, San Diego, CA). The magnitudes of \( I_o \), \( V_e \), and voltage changes associated with current pulses were digitized (DT 2801; Data
Translation, Marlboro, MA) and stored on an IBM PC-XT computer. Transepithelial conductance (G_e) was calculated using terminated cable equations as described previously (Hebert et al., 1981b; Hebert and Andreoli, 1986).

**Measurement of transcellular conductance (G_c).** In the absence of NH_4^+ apical membranes of the mouse MTAL are predominantly, if not exclusively, conductive to potassium via K^+ channels (Hebert and Andreoli, 1984; Hebert, Friedman, and Andreoli, 1984). Recent patch clamp studies have demonstrated that this K^+ channel is inhibited by Ba^{2+} (Bleich, Schlatter, and Greger, 1990; Wang, White, Geibel, and Giebisch, 1990), and we have previously shown that the apical K^+ conductive pathway in mouse IP-MTAL can be completely blocked by the addition of 20 mM luminal Ba^{2+} in the absence of luminal K^+ (Hebert and Andreoli, 1986). Thus, the magnitude of G_e observed in the presence of 20 mM Ba^{2+}/0 K^+ in the luminal medium provides an estimate of the transepithelial shunt (paracellular) conductance (G_e), and the difference between G_e observed with 0 mM Ba^{2+}/5 mM K^+ vs. 20 mM Ba^{2+}/0 mM K^+ (G_e^{20Ba/0K} - G_e^{0Ba/0K}) provides a good approximation of G_c, the transcellular conductance (Hebert and Andreoli, 1986).

\[
G_e = G_c + G_s \tag{2a}
\]

\[
G_c = G_e^{20Ba/0K} \tag{2b}
\]

\[
G_c = G_e - G_e^{20Ba/0K} \tag{2c}
\]

The luminal Ba^{2+}-sensitive G_c (or G_s) in the IP-MTAL was used to indirectly assess the activity of apical K^+ channels. This method was used because of the difficulty in obtaining adequate long-term microelectrode impalements required for the experimental protocols used. Changes in G_c have been used previously by us to estimate changes in apical and basolateral conductive pathways (Hebert and Andreoli, 1986; Molony and Andreoli, 1988). In this study, all the electrical experiments were performed in the presence of arginine vasopressin (AVP; 10 μU/ml; ~5 × 10^{-11} M) in the bathing medium. This concentration of AVP produces a maximal increase in G_c without affecting G_s (Hebert and Andreoli, 1984). The AVP-induced increase in apical G_c allowed us to detect small degrees of inhibition in G_c. Standard CO_2/HCO_3^- free perfusing and bathing media used in the electrical experiments were identical to those used in the pH_experiments.

**Drugs and Reagents**

BCECF-AM was obtained from Molecular Probes, Inc. (Eugene, OR). All other agents were obtained from Sigma Chemical Co. (St. Louis, MO), and were of analytical grade.

**Statistics**

Results on a single S-MTAL preparation or IP-MTAL constituted a single n. Unless stated, each experimental maneuver was repeated on at least three separate S-MTAL preparations or IP-MTALs. All experimental results are expressed as means ± SE. The Student's t test was used to analyze paired data, while ANOVA was used to evaluate unpaired groups; P < 0.05 was considered significant.

**RESULTS**

**Cellular Buffering Power**

Fig. 2 shows a plot of the intrinsic buffering power (β; millimolar per pH unit) of mouse MTAL cells over the pH_i range 6.5–7.6. β was measured in S-MTAL as (Roos
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and Boron, 1981; Boyarsky et al., 1988):

\[ \beta_i = -\frac{\Delta A}{\Delta pH} \]  \hspace{1cm} (3)

where \( \Delta A \) is the millimolar amount of acid added and \( \Delta pH \) is the resultant drop in pH. The initial intracellular pH (segment a-b) was varied by altering extracellular pH (pH\textsubscript{o}) from 6.4 to 7.9. Known intracellular proton loads were then acutely delivered to MTAL cells at point b by either the abrupt removal of extracellular NH\textsubscript{4}Cl (5 mM, pK = 9) or the abrupt addition of extracellular sodium acetate (10 mM, pK = 4.75), and the acute drops in pH\textsubscript{i} (segment b-c) were monitored (Roos and Boron, 1981; Zeidel, Silva, and Seifter, 1986; Boyarsky et al., 1988). The acetate addition protocol was used to deliver acute proton loads (Zeidel et al., 1986) at pH\textsubscript{i} levels > 7.1, since the pH\textsubscript{i} of S-MTAL in NH\textsubscript{4}\textsuperscript{+}-containing medium was < 7.1. To prevent pH\textsubscript{i} regulation by Na\textsuperscript{+}:H\textsuperscript{+} exchangers and HCO\textsubscript{3}\textsuperscript{-} transporters (Kikeri et al., 1990a), experiments were performed in CO\textsubscript{2}/HCO\textsubscript{3}\textsuperscript{-}-free and Na\textsuperscript{+}-free medium containing amiloride.

It is evident from Fig. 2 that the intrinsic buffering power remained relatively stable over a pH\textsubscript{i} range of 7.0-7.6 (\( \beta_i = 29.7 \pm 2.4 \) mM/pH\textsubscript{i} at pH\textsubscript{i} of 7.22 \pm 0.08, \( n = 8 \); slope not significantly different from zero), but gradually increased with cell acidification below a pH\textsubscript{i} of 7.0 (\( \beta_i = 50 \) mM/pH\textsubscript{i} at pH\textsubscript{i} 6.5). An inverse relationship between \( \beta_i \) and pH\textsubscript{i} has been previously described in other cell types by us (Kikeri, Zeidel, Ballermann, Brenner, and Hebert, 1990b) and others (Roos and Boron, 1981; Boyarsky et al., 1988). In addition, the observed values for \( \beta_i \) in MTAL cells are similar to those reported for proximal tubule cells (43 mM/pH\textsubscript{i} [Krapf, Alpern, Rector, and Berry, 1987]) and white blood cells (28 mM/pH\textsubscript{i} [Grinstein and Furuya, 0.1-0.5 mM). In addition, we assumed that [NH\textsubscript{3}\textsubscript{i}] = [NH\textsubscript{3}\textsubscript{o}] and [acetic acid]\textsubscript{i} = [acetic acid]\textsubscript{o} in the presence of extracellular NH\textsubscript{4}\textsuperscript{+} and acetate, respectively. The very slow rate of pH\textsubscript{i} recovery after either extracellular NH\textsubscript{4}\textsuperscript{+} removal or extracellular acetate addition (Fig. 2, insert, segment c-d) indicated that acid extrusion or acetate\textsuperscript{-} entry was negligible in the absence of extracellular Na\textsuperscript{+}.

FIGURE 2. Intrinsic buffering power of MTAL cells. Open circles, NH\textsubscript{4}Cl withdrawal; solid circles, sodium acetate addition; open square, addition of NH\textsubscript{4}Cl in the presence of 10 mM Ba\textsuperscript{2+}, 1 mM furosemide, and 5 mM ouabain (see text for details). (Insert) Effect of NH\textsubscript{4}Cl withdrawal. Experiments were performed in the absence of extracellular Na\textsuperscript{+} (Na\textsuperscript{+} replaced with N-methyl-D-glucamine\textsuperscript{+}) in the presence of 0.1-0.5 mM amiloride. Segment a-b represents pH\textsubscript{i} in medium containing 5 mM NH\textsubscript{4}Cl, pH\textsubscript{o} 7.44. At b, extracellular NH\textsubscript{4}Cl was abruptly withdrawn.
but are approximately three- to sixfold greater than those reported for smooth muscle cells (9 mM/pH in A10 cells at pH 7.2 [Kikeri et al., 1990b]; 9 mM/pH at pH 7.0 [Aickin, 1984]) or glomerular mesangial cells (5 mM/pH at pH 7.3 [Boyarsky et al., 1988]).

In additional experiments (n = 5) we estimated $B_i$ at pH levels > 7.1 pH units by adding 5 mM NH$_4$Cl to S-MTAL (in standard Na+-containing, CO$_2$/HCO$_3$-free medium, pH$_o$ 7.4) in the presence of inhibited cellular NH$_4^+$ transport (i.e., in the presence of the combination of 10 mM barium, 1 mM furosemide, and 5 mM ouabain [Kikeri et al., 1989]), and measuring the acute increase in pH$_i$. Values of $B_i$ obtained under these conditions (30.9 ± 3.0 mM/pH$_i$ at a mean pH$_i$ of 7.48 ± 0.01, n = 5, Fig. 2, open square) were similar to those obtained using acetate addition or NH$_4^+$ withdrawal at pH$_i$ levels > 7.0.

Cellular NH$_4^+$ Entry Pathways in Mouse MTAL

Addition of NH$_4^+$ to the luminal perfusate in the IP-MTAL leads to a prompt cell acidification due to NH$_4^+$ influx across the NH$_3$-impermeable apical membrane, and this NH$_4^+$-mediated fall in pH$_i$ can be abolished by prior exposure of the apical membrane to the combination of luminal 0.1 mM furosemide and 10-20 mM luminal Ba$^{2+}$ (Kikeri et al., 1989). In these experiments we assessed the relative contribution of each of these two pathways to NH$_4^+$ entry across apical membranes of the IP-MTAL. Rates of cell acidification due to luminal addition of 0.1 mM NH$_4^+$ were measured before and 3-5 min after addition of either 0.1 mM furosemide or 10 mM Ba$^{2+}$ to luminal perfusate. Under these conditions, which mimic concentrations of NH$_4^+$ observed in vivo, [NH$_4^+$], would be negligible due to the high NH$_4^+$ permeability of the basolateral membrane and the absence of basolateral medium NH$_4$Cl. Consequently, we assumed that $B_i = B_r$. Moreover, since acid extrusion (predominantly via apical Na$^+$:H$^+$ exchange [Kikeri et al., 1990a] and acid loading are equal at the steady-state pH$_i$, the initial rate of H$^+$ influx (calculated according to Eq. 1) on addition of luminal NH$_4$Cl reflects the initial rate of cellular NH$_4^+$ influx.$^1$ In four IP-MTAL tubules, addition of 0.1 mM NH$_4$Cl to K$^+$-free luminal medium at the steady-state pH$_i$ (7.38 ± 0.11, n = 4) resulted in an initial NH$_4^+$ influx rate of 21.7 ± 3.3 mM/min and this NH$_4^+$ influx was completely blocked by addition of both 0.1 mM furosemide and 10 mM Ba$^{2+}$ to the luminal medium. 0.1 mM apical furosemide inhibited apical NH$_4^+$ entry by 55.3 ± 6.1% (11.7 ± 1.9 mM/min) and 10 mM apical Ba$^{2+}$ inhibited apical NH$_4^+$ entry by 44.8 ± 6.1% (10 ± 2.9 mM/min). Thus in the absence of inhibitors, both the apical Ba$^{2+}$-sensitive pathway and Na$^+$:K$^+$:NH$_4^+$:2Cl$^-$ cotransporter mediated significant apical entry of NH$_4^+$ into mouse MTAL cells at ammonium concentrations observed in vivo.

Fig. 3 shows the initial rate of apical NH$_4^+$ entry in IP-MTAL, via both the Ba$^{2+}$- and furosemide-sensitive pathways, after addition of NH$_4$Cl to luminal fluid at concentrations from 0.1 to 20 mM (n = 8 tubules). Steady-state pH$_i$ in NH$_4^+$-free medium

$^1$This was substantiated by our previous observation that removal of luminal NH$_4$Cl (in the absence of basolateral NH$_3$Cl) results only in a small cell acidification even in the absence of pH$_i$ regulation (Kikeri et al., 1989).
averaged 7.26 ± 0.04 pH units. In these experiments, media contained 100 mM Na⁺ and 40 mM N-methyl-D-glucamine⁺ (NMDG⁺); NH₄⁺ replaced NMDG⁺ in an equimolar manner. The apparent $K_{0.5}$ and the maximal rate of luminal NH₄⁺ entry were 0.5 and 83.3 mM/min, respectively. This low $K_{0.5}$ value may be due both to the high affinity of NH₄⁺ for the Na⁺:K⁺(NH₄⁺):2Cl⁻ cotransporter (Kinne et al., 1986) and to inhibition of both apical NH₄⁺ entry mechanisms by factors such as pH (this possibility is evaluated in the experiments described below).

To determine if NH₄⁺ could enter MTAL cells via the basolateral Na⁺:K⁺-ATPase, ouabain-sensitive acidification was evaluated in S-MTAL in the presence of 0.5–20 mM NH₄Cl ($n = 5$). Steady-state pHᵢ of S-MTAL in NH₄⁺-free medium averaged 7.42 ± 0.02 pH units ($n = 16$). In the experiments shown in Fig. 4, which were performed on a representative S-MTAL preparation, segments a-b represent steady-state pHᵢ of S-MTAL in NH₄⁺-free medium, pHᵢ 7.4. 5 mM NH₄Cl was then added to the extracellular medium at point b. The lower trace shows the control acidification response after addition of 5 mM NH₄Cl to the medium bathing apical and basolateral membranes of S-MTAL (b-g). However, addition of 5 mM NH₄Cl in the presence of 10 mM Ba²⁺, 1 mM furosemide, and 5 mM ouabain resulted in rapid cell alkalinization due to entry of NH₃ across NH₃-permeable basolateral membranes (Kikeri et al., 1989; compare c-d with b-g). Thus, the combination of Ba²⁺, furosemide, and ouabain inhibited virtually all the entry of NH₄⁺ into MTAL cells. The pHᵢ on addition of NH₄Cl in the presence of inhibited NH₄⁺ transport was similar to the pHᵢ expected if cell membranes were permeable only to NH₃. In other words, inhibition of NH₄⁺ transport by the combination of barium plus furosemide and ouabain converted the

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$\frac{1}{\text{Rate}} = 0.006\left(\frac{1}{[\text{NH}_4^+]}\right) + 0.012; \ K_{0.5} = 0.5 \text{ mM}; \ \text{maximal rate of influx} = 83.3 \text{ mM/min}$.

The maximal rate of luminal NH₄⁺ entry of 83.3 mM/min can be converted to more conventional transport units, assuming a MTAL tubule inner diameter of 20 μm and a MTAL tubule cell volume of 0.25 nl/mm tubule length (Hebert, 1986). Using these parameters, $\frac{\text{nmol}}{\text{min} \cdot \text{mm}} = 20 \text{ pmol/min-mm} \text{ or } 550 \text{ pmol/s-cm}^2$. These rates are consistent with rates of net NH⁺ absorption observed in perfused thick ascending limb tubules (Knepper et al., 1989).
highly NH\textsubscript{3}-permeable, native S-MTAL cells into almost purely NH\textsubscript{3}-permeable cells (across basolateral membranes). The selective removal of ouabain resulted in partial restoration of the acidification response to 5 mM NH\textsubscript{4}Cl addition (e-f). The ouabain-sensitive rate of NH\textsubscript{i} entry with 20 mM extracellular NH\textsubscript{4}Cl was 17.4 ± 0.4 mM/min (calculated as initial rates [e-f] − [c-d]; n = 3). In separate experiments performed in the absence of NH\textsubscript{4}Cl, the addition of either the combination of Ba\textsuperscript{2+}, furosemide, and ouabain or the combination of Ba\textsuperscript{2+} and furosemide to S-MTAL did not alter steady-state pH\textsubscript{i} over 200 s (ΔpH\textsubscript{i} = 0.01 ± 0.02, n = 2). Thus, the basolateral, ouabain-sensitive Na\textsuperscript{+}:K\textsuperscript{+}-ATPase could mediate NH\textsubscript{i} entry into MTAL cells.

Effect of pH\textsubscript{i} on Total Rate of NH\textsubscript{i} Entry via Ba\textsuperscript{2+}-, Furosemide-, and Ouabain-sensitive Pathways in S-MTAL

Fig. 5 illustrates the effect of pH\textsubscript{i} on the total rate of cellular NH\textsubscript{i} entry in a representative S-MTAL preparation, i.e., via the combination of the Ba\textsuperscript{2+}-sensitive NH\textsubscript{i} transport pathway, the furosemide-sensitive Na\textsuperscript{+}:K\textsuperscript{+}(NH\textsubscript{4})\textsubscript{2}Cl\textsuperscript{-} cotransporter,
and the ouabain-sensitive Na⁺:K⁺(NH₄⁺)-ATPase. Segment a-b represents steady-state pHe of S-MTAL in NH₄⁺-free medium (7.45 ± 0.05, n = 3). Addition of 5 mM NH₄Cl at b reduced pHe to a new steady-state level (7.13 ± 0.01, c-d). At d, either amiloride (1 mM) or vehicle (DMSO) was added to the medium. The gap in the trace represents a 2–4-min incubation period.

Abrupt inhibition of total NH₄⁺ entry at point f, in the absence of amiloride, by adding the combination of 10 mM Ba²⁺, 1 mM furosemide, and 5 mM ouabain resulted in rapid pHe recovery (f-g). Since net rates of NH₄⁺ entry (via the combination of Ba²⁺-, furosemide-, and ouabain-sensitive pathways) and NH₄⁺ exit (by proton extrusion via Na⁺:H⁺ exchange coupled with NH₄⁺ diffusion across the basolateral membrane [Kikeri et al., 1989]) are probably equal during the steady state in NH₄⁺-containing medium (e-f), the initial rate of acid extrusion at point f (initial pHᵢ recovery rate × βₖ, where βₖ = βₙᵢ + βₙH₄) of 33.7 ± 3.8 mM/min equals the total rate of ammonium entry (via the Ba²⁺-, furosemide-, and ouabain-sensitive pathways) during segment e-f.

Amiloride addition in the presence of 5 mM ambient NH₄Cl at point d reduced pHe within 1 min, because of inhibition of Na⁺:H⁺ exchange (Kikeri et al., 1990a), to a new steady-state level of 6.96 ± 0.02 (h-i). As discussed above, NH₄⁺ entry and exit are equal during segment h-i. Inhibition of total NH₄⁺ entry with the combination of Ba²⁺, furosemide, and ouabain at point i led to a markedly reduced rate of acid extrusion (5.9 ± 0.07 mM/min). Since both apical and basolateral membranes of S-MTAL are exposed to NH₄Cl, and because basolateral membrane are highly permeable to NH₃, [NH₄⁺]i would increase as pHe decreases. Increases in [NH₄⁺]i, in turn, would reduce the chemical gradient favoring NH₄⁺ uptake. Thus the combined effects of the reduction of pHe from 7.13 ± 0.01 (e-f) to 6.96 ± 0.02 (h-i) and the associated rise in [NH₄⁺]i, reduced the total rate of cellular NH₄⁺ entry across both apical and basolateral membranes by > 80%.³ In addition, these data indicate that Na⁺:H⁺ exchange plays the dominant role in NH₄⁺ exit (H⁺ extrusion coupled to NH₃ diffusion [Kikeri et al., 1989]) from mouse MTAL cells in the presence of ambient NH₄Cl.

Effect of pHₒ/pHᵢ on Transcellular Conductance (Gₒ) in IP-MTAL

Because of the difficulty in obtaining long-term microelectrode impalements in the small epithelial cells of the mouse MTAL and the necessity of obtaining paired data (because of large tubule-to-tubule variations in transepithelial conductance [Hebert

³ Amiloride does not appear to significantly affect any of the NH₄⁺ entry processes. We (Kikeri et al., 1989) have shown that the combination of luminal 10 mM Ba²⁺ and luminal 0.1 mM furosemide completely abolishes the cell acidification observed with luminal 20 mM NH₄⁺ addition in IP-MAL and that these two agents plus 5 mM ouabain completely block NH₄⁺-induced cell acidification in S-MAL. In addition, in this paper (Fig. 3) we demonstrate that inhibition of the Na⁺:H⁺ exchanger (Na⁺ replaced by NMDG⁺) does not affect luminal NH₄⁺-induced cell acidification in IP-MAL. Furthermore, we have previously shown (Fig. 4 in Kikeri et al., 1990a) that amiloride has no significant effect on Q₀ₙ, a sensitive index to the activity of both the apical Na⁺:K⁺/NH₄⁺;2Cl⁻ entry mechanism and the basolateral Na⁺,K⁺-ATPase exit mechanism. Finally, if amiloride had a significant effect on the K⁺ channel then the cell would depolarize (for example, luminal Ba²⁺ depolarizes the mouse MTAL cell; Hebert and Andreoli, 1984) and the cell would be expected to alkalinize somewhat because the driving force for H⁺ entry would be reduced.
and Andreoli, 1984, 1986]), we used an alternative approach to estimate the effects of $pH_l/pH_h$ on the apical $Ba^{2+}$-sensitive $K^+$ conductance using measurements of transcellular conductance ($G_c$). As described in detail in Methods (see Eqs. 2a–2c), the difference between transepithelial conductance ($G_t$) measurements observed in IP-MTAL with perfusate containing either $0 \text{mM } Ba^{2+}/5 \text{ mM } K^+$ (total $G_t$) or $20 \text{ mM } Ba^{2+}/0 \text{ mM } K^+$ (shunt conductance, $G_s$) provides a good approximation of the transcellular conductance, $G_c$. This approach has provided valuable information on the regulation of cellular conductive pathways in the MTAL in previous studies (Hebert and Andreoli, 1984, 1986; Molony and Andreoli, 1988).

$G_c$ decreased from $110.9 \pm 4.4$ to $40.4 \pm 1.2 \text{ mS/cm}^2$ ($n = 4; P < 0.01$) on changing the luminal medium from $0 \text{ mM } Ba^{2+}/5 \text{ mM } K^+$ to $20 \text{ mM } Ba^{2+}/0 \text{ mM } K^+$ at a constant luminal medium $pH$ of 7.4, indicating that $G_c$ and $G_t$ accounted for approximately two-thirds and one-third of $G_c$, respectively. Similar $G_t:G_c$ ratios were also observed in previous studies (Hebert and Andreoli, 1984, 1986). In another set of experiments ($n = 4$) the luminal medium $pH$ ($pH_{lum}$) was reduced in a stepwise manner from $pH$ 7.4 to $pH$ 3.0 in the presence of luminal $0 \text{ mM } Ba^{2+}/5 \text{ mM } K^+$. The solid squares in Fig. 6 show the relative changes in $G_c$ normalized such that $G_c$ at $pH_{lum}$ 7.4 was arbitrarily set at 1. Changing the luminal medium from $0 \text{ mM } Ba^{2+}/5 \text{ mM } K^+$ to $20 \text{ mM } Ba^{2+}/0 \text{ mM } K^+$ at a constant $pH_{lum}$ of 4 did not alter $G_c$ ($G_{c \text{ 20um}} = 22.3 \pm 5.7; G_{c \text{ 0um}} = 31.9 \pm 11.1; \Delta G_c = -9.6 \pm 5.4, n = 4, \text{ NS}$). Therefore, reducing $pH_{lum}$ from 7.4 to 4.0 abolished virtually all the cell conductance but had no significant effect on $G_c$. These data demonstrate indirectly that the apical $Ba^{2+}$-sensitive $K^+$ conductance was sensitive to luminal medium $pH$.

**Figure 6.** Effect of $pH_{lum}$ and $pH_h$ on relative $Ba^{2+}$-sensitive cell conductance ($G_c$) in IP-MTAL (see Methods and Eqs. 2a–2c for procedure for assessing $G_c$). The solid squares and dashed line show the relationship between $pH_{lum}$ and relative $G_c$, while the solid triangles show the relationship between $pH_h$ and relative $G_c$. The relation

$$\text{relative rate} = A + B \left[ \frac{10^{(pH_{lum} - pK)}}{1 + 10^{(pH_{lum} - pK)}} \right]$$

where $A$ and $B$ are the intercept and slope, respectively, was fit to the data. $pK = 6.7 (r = 0.95)$ for the $pH_h$ fit.
The experiments shown in Fig. 7 were performed to determine the effect of \( \text{pH}_{\text{lu}} \) on \( \text{pH}_{i} \) in the IP-MTAL \((n = 4)\). Using experimental conditions identical to those used in the electrical experiments, \( \text{pH}_{\text{lu}} \) was reduced in a stepwise manner from \( \text{pH} 7.4 \) to \( \text{pH} 5.0 \) (luminal medium contained 0 mM \( \text{Ba}^{2+} / 5 \) mM \( \text{K}^{+} \)) and \( \text{pH}_{i} \) was monitored at each luminal medium \( \text{pH} \) level. As shown in Fig. 7, \( \text{pH}_{i} \) was greater than luminal medium \( \text{pH} \) at acidic \( \text{pH}_{i} \) levels, possibly because of \( \text{pH}_{i} \) regulation by \( \text{Na}^{+}:\text{H}^{+} \) exchange, while \( \text{pH}_{i} \) was less than luminal medium \( \text{pH} \) at medium \( \text{pH} \) levels > 7.5 \( \text{pH} \) units. This relationship between luminal medium \( \text{pH} \) and \( \text{pH}_{i} \) shown in Fig. 7 was then used to determine the relationship between \( \text{pH}_{i} \) and relative apical \( \text{Ba}^{2+} \)-sensitive transcellular conductance (solid triangles, Fig. 6). As shown in Fig. 6, the relative \( G_{c} \) was related, over a \( \text{pH}_{i} \) range of 6.0–7.4, to \( \text{pH}_{i} \), with 50% inhibition at a \( \text{pH}_{i} \) of ~6.7 and/or to luminal \( \text{pH} \), with a 50% inhibition at \( \text{pH}_{o} \) ~5.5.

![Figure 7. Effect of \( \text{pH}_{\text{lu}} \) on \( \text{pH}_{i} \) in IP-MTAL. The line is the least-squares fit of the data: \( y = 0.442x + 4.26, r = 0.98 \).](image)

*Effect of \( \text{pH} \), on \( \text{NH}_{4}^{+} \) Entry due to Apical \( \text{Ba}^{2+} \)-sensitive \( \text{NH}_{4}^{+} \) Transport in IP-MTAL*

Since \( \text{pH}_{i} \) in the electrical experiments shown in Fig. 6 was altered by changing luminal medium \( \text{pH} \), either luminal medium \( \text{pH} \) (\( \text{pH}_{\text{lu}} \)) or \( \text{pH}_{i} \) may have been responsible for the changes in \( G_{c} \). Moreover, it is possible that the \( \text{pH} \)-dependent changes in \( G_{c} \) resulted from alterations in either apical or basolateral conductances, or both. We therefore assessed the effects of \( \text{pH}_{o} \), independent of luminal \( \text{pH} \), changes, on \( \text{NH}_{4}^{+} \) entry via the apical \( \text{Ba}^{2+} \)-sensitive \( \text{NH}_{4}^{+} \) transport pathway in IP-MTAL \((n = 5)\). The results of these experiments are shown in Fig. 8.

The rate of change in \( \text{pH}_{i} \) was measured after addition of 1 mM \( \text{NH}_{4} \text{Cl} \) to a luminal medium in which both \( \text{Na}^{+} \) and \( \text{K}^{+} \) were replaced isosmotically with \( \text{NMDG}^{+} \) and to which 0.1 mM furosemide was added. Under these conditions the \( \text{H}^{+} \) influx due to \( \text{NH}_{4}^{+} \) entry represents exclusively the apical \( \text{Ba}^{2+} \)-sensitive \( \text{NH}_{4}^{+} \) transport pathway. The initial rate of \( \text{NH}_{4}^{+} \) influx, \( J_{\text{NH}_{4}^{+}} \), was then calculated according to Eq. 1. In addition, the removal of luminal \( \text{Na}^{+} \) would abolish any \( \text{H}^{+} \) efflux mediated by the apical \( \text{Na}^{+}:\text{H}^{+} \) exchanger. For these experiments we also assumed that \( \beta_{i} \approx \beta \), since intracellular \( \text{NH}_{4}^{+}/\text{NH}_{3} \) concentrations would be low due to the absence of basolateral \( \text{NH}_{4} \text{Cl} \) and the high basolateral membrane \( \text{NH}_{3} \) permeability (Kikeri et al., 1989).

To assess the effect of \( \text{pH}_{i} \) on the \( \text{Ba}^{2+} \)-sensitive (i.e., furosemide-insensitive) apical \( J_{\text{NH}_{4}^{+}} \), the \( \text{pH}_{i} \) (before luminal \( \text{NH}_{4}^{+} \) addition) was altered by changing the \( \text{pH} \) of the
basolateral bath solution in stepwise fashion over the range 4-8. The relationship between basolateral pHo and pHi was virtually identical to that observed for apical pHo and pHi (Fig. 7). As shown in Fig. 8, the relative rate of furosemide-insensitive, apical NH4+ entry, normalized to the influx rate obtained at pHi ~ 7.3, was dependent on pHi in IP-MTAL tubules with reductions in pHi inhibiting the apical NH4+ flux. At pHi values < 6.5, JNH was inhibited > 80% compared with JNH values at pHi > 7.5. In four additional IP-MTAL tubules, pHi was reduced with luminal amiloride (0.5 mM) in the presence of luminal Na+ and 0.1 mM furosemide (apical and basolateral pHo 7.40; Fig. 8, open squares). Inhibition of apical Na+:H+ exchange resulted in a decrease in pHi of ~ 2.6 ± 0.03 pH units and a fall in relative JNH of 15.3 ± 3.5% (Fig. 8, open squares), a value that was indistinguishable from the relative reduction observed when pHi was decreased by changing basolateral pHo. The results in Fig. 8 demonstrate that reducing pHi by variations in basolateral pH or by addition of luminal amiloride (at constant pHi) inhibited NH4+ influx via the apical Ba2+-sensitive NH4+ transport pathway. The observations in Figs. 6-8 that reductions in pHi to similar values by decreasing luminal or basolateral pHo or by luminal amiloride addition at a constant pHo led to similar fractional reductions in Ba2+-sensitive NH4+ entry (or K+ conductance) suggests that cell pH rather than pHo was the major factor affecting the Ba2+-sensitive entry mechanism.

**Effect of pH on NH4+ Entry due to Ba2+-sensitive NH4+ Transport in S-MTAL**

**Determination of rate of NH4+ entry due to Ba2+-sensitive NH4+ pathway.** Fig. 9 shows in a representative experiment on a single S-MTAL preparation (a) the control acidification response on addition of 5 mM NH4Cl at point b to S-MTAL (b-f), (b) the effect of 1 mM furosemide plus 5 mM ouabain on the acidification response (b-e), and (c) the effect of 10 mM Ba++, furosemide, and ouabain on the pHi response to NH4Cl addition (b-c-d). Addition of either furosemide plus ouabain or Ba++, furosemide plus ouabain in the absence of NH4Cl did not alter steady-state pHi (n = 2). As shown previously in Fig. 4 (upper curve), addition of extracellular NH4Cl when NH4+ was set to 1.0. Open squares, experiments with and without luminal 0.5 mM amiloride (to inhibit the apical Na+:H+ antiporter) and in the presence of luminal Na+ (n = 5). The solid line is a least-squares linear fit: y = 16.23x - 106.16 (r = 0.89).
transport was completely inhibited (i.e., in the presence of the combination of Ba$^{2+}$, furosemide, and ouabain) resulted in virtually instantaneous (< 2 s) alkalinization (b-c; $\Delta pHi = 0.16 \pm 0.02$, $n = 5$). In either the presence of furosemide plus ouabain (middle curve) or the absence of inhibitors (lower curve), sustained, rapid acidification was observed. The initial rate of decrease in pHi in the presence of furosemide plus ouabain was taken to represent the initial rate of NH$_4^+$ influx via Ba$^{2+}$-sensitive NH$_4^+$ transport pathways. $J_{NH}$ was calculated according to Eq. 1, where $\beta_i = \beta_a + \beta_{NH}$. Since both apical and basolateral membranes in S-MTAL are exposed to ambient NH$_4$Cl, the observed $J_{NH}$ values represent Ba$^{2+}$-sensitive NH$_4^+$ transport across both apical and basolateral membranes. Apical membranes of MTAL cells possess an apical Ba$^{2+}$-sensitive NH$_4^+$ transport pathway (Kikeri et al., 1989); whether a Ba$^{2+}$-sensitive NH$_4^+$ transport pathway exists on basolateral membranes of MTAL cells is unknown.

**Effect of pH$_i$ on NH$_4^+$ entry due to Ba$^{2+}$-sensitive NH$_4^+$ transport.** The experiments shown in Fig. 10 were used to assess NH$_4^+$ entry via the Ba$^{2+}$-sensitive pathways in S-MTAL at various pH$_i$ values using the strategy discussed above (Fig. 9). Addition of 5 mM NH$_4$Cl to the medium bathing S-MTAL at the resting pH$_i$ resulted in a rapid
fall in pH at 7.41 ± 0.02 (~2 s after addition of NH4Cl; point b), the initial rate of Ba2+-sensitive NH4+ entry in the presence of 5 mM NH4Cl averaged 32.6 ± 3.1 mM/min (n = 7). Addition of amiloride to S-MTAL reduced pH from 7.43 ± 0.04 to 7.20 ± 0.03 within 1 min (n = 4, P < 0.05). The subsequent addition of 5 mM NH4Cl to amiloride-treated cells (Fig. 10 B, point b) resulted in a small increase in pH to 7.26 ± 0.02 (segment b-c) followed by a much larger fall in pH (segment c-d). The small rise in pH with exposure to NH4Cl suggests that the initial rate of H+ entry due to Ba2+-sensitive NH4+ transport at the acidic pH of 7.20 ± 0.03 was reduced relative to the rate of buffering of H+ due to the rapid entry of NH4+ across the basolateral membrane. At a pH of 7.26 ± 0.02, the initial rate of Ba2+-sensitive NH4+ entry averaged 18.8 ± 1.2 mM/min, a 42% reduction compared with the influx rate at pH 7.41 (n = 4).

Fig. 11 shows the direct relationship between pH and the initial rate of Ba2+-sensitive NH4+ entry in S-MTAL. Decreases in Ba2+-sensitive flow were observed both with the spontaneous variations in resting pH (open circles) and with the further reductions in pH resulting from exposure to amiloride (solid circles). When the results in Fig. 11 are taken together with those in Figs. 8 and 6, it is clear that decreasing pH over the physiological range of 6.0–7.8 dramatically reduces the activity of the Ba2+-sensitive K+/NH4+ pathway in apical membranes.

Effect of pH on NH4+ Entry by Apical Na+:K+/NH4+:2Cl− Cotransport in IP-MTAL

Experiments were performed in IP-MTAL to determine if NH4+ entry into MTAL cells via the apical Na+:K+(NH4+):2Cl− cotransporter (Kinne et al., 1986; Kikeri et al., 1989) was sensitive to pH. Fig. 12 shows representative experiments in IP-MTAL illustrating the effects of initial steady-state pH on the rate of acidification induced by addition of 20 mM NH4Cl to K+-free perfusate in the presence of 20 mM BaCl2. Since the combination of furosemide (0.1 mM) and Ba2+ (10–20 mM) in the luminal medium inhibits all the apical NH4+ entry in IP-MTAL, the fall in pH observed on addition of luminal NH4Cl in the presence of luminal Ba2+ represents NH4+ entry via the apical Na+:K+(NH4+):2Cl− cotransporter. Luminal NH4Cl was added at points a, c, e, and g. Segments a-b and c-d are the acidification responses in two tubules at the different spontaneous, initial, steady-state pH values a and c. Addition of 0.5 mM
amiloride to the luminal fluid in the absence of NH₄Cl resulted in acidification from a to e and from c to g in these two tubules: the average amiloride-induced drop in pHᵢ was 0.49 pH units (n = 4). The data in Fig. 12 clearly demonstrate that as the initial steady-state pHᵢ is reduced, the rate of cellular acidification mediated by luminal NH₄⁺ influx via the cotransporter is considerably slowed.

Fig. 13 shows the direct relationship between the initial steady-state pHᵢ and the initial rate of NH₄⁺ entry via apical Na⁺:K⁺(NH₄⁺):2Cl⁻ cotransporter. Note that the open squares show the usual spontaneous variability of resting pHᵢ in the absence of amiloride these spontaneous variations in pHᵢ correlated closely with the observed rate of ammonium entry. The amiloride-mediated reduction in pHᵢ from 7.31 ± 0.17 to 6.8 ± 0.14 pH units was associated with a 52.9 ± 13.4% decrease in the rate of NH₄⁺ influx via the Na⁺:K⁺(NH₄⁺):2Cl⁻ cotransporter. Thus, apical NH₄⁺ entry via the furosemide-sensitive cotransporter, like that through the Ba²⁺-sensitive apical pathway, was markedly sensitive to pHᵢ.

It should be noted that the decrease in pHᵢ in IP-MTAL with luminal amiloride addition in the presence of luminal Ba²⁺ (0.49 pH units) was larger than that observed in the presence of furosemide (0.26 pH units in IP-MTAL; 0.23 pH units in S-MTAL). Although the reason for this difference was not evaluated further in this study, it is possible that reduction of metabolic acid production because of inhibition of salt transport-related energy consumption by furosemide (in the MTAL, furosemide-sensitive oxygen consumption accounts for > 50% of the total rate of oxygen consumption [Kikeri et al., 1990]) may partially explain this difference.
DISCUSSION

The results of this study demonstrate that (a) NH$_4^+$ is transported at high affinity and at high rates across apical membranes of MTAL cells via a Ba$^{2+}$-sensitive NH$_4^+$ transport pathway and Na$^+$.K$^+$(NH$_4^+$):2Cl$^-$ cotransport; (b) NH$_4^+$ entry via both the Ba$^{2+}$-sensitive NH$_4^+$ transport pathway and Na$^+$.K$^+$(NH$_4^+$):2Cl$^-$ cotransport was sensitive to pH$_i$ over a pH$_i$ range of 6.8-7.2 pH units; (c) the effect of pH$_i$ on NH$_4^+$ entry via the Ba$^{2+}$-sensitive pathway was greater in S-MTAL (42% reduction on decreasing pH$_i$ from 7.41 to 7.26) than in IP-MTAL (15% reduction on decreasing pH$_i$ from 7.27 to 7.01), suggesting a modulatory effect of NH$_3$/NH$_4^+$; and (d) NH$_4^+$ could enter MTAL cells via the basolateral Na$^+$.K$^+$-ATPase, i.e. Na$^+$.K$^+$(NH$_4^+$)-ATPase.

NH$_4^+$ Transport by the Ba$^{2+}$-sensitive Pathway and Na$^+$.K$^+$(NH$_4^+$):2Cl$^-$ Cotransport

In mouse MTAL cells ~45% of the initial rate of apical NH$_4^+$ entry occurred via the Ba$^{2+}$-sensitive pathway while 55% was mediated by Na$^+$.K$^+$(NH$_4^+$):2Cl$^-$ cotransport. Apical membranes of mouse MTAL cells also possess a Na$^+$.H$^+$ exchanger (Kikeri et al., 1990a); epithelial Na$^+$.H$^+$ exchangers can transport NH$_4^+$ (Kinsella and Aronson, 1981). However, the NH$_4^+$-induced acidification rate in S-MTAL was reduced by 98.5 ± 0.6% ($n = 7$) by the combination of Ba$^{2+}$, furosemide, and ouabain (compare segments c-d and b-g in Fig. 4, and segments c-d and b-f in Fig. 9). Moreover, the combination of luminal Ba$^{2+}$ and furosemide in the IP-MTAL completely abolished the acidification observed on adding luminal NH$_4^+$ (Kikeri et al., 1989). Thus, the apical Na$^+$.H$^+$ exchanger in the mouse MTAL does not appear to mediate significant entry of NH$_4^+$ ions when compared with the entry attributable to the combination of the Ba$^{2+}$-sensitive pathway and the furosemide-sensitive cotransporter.

In the isolated perfused rat TAL, Good et al. (1984) found that luminal furosemide virtually abolished transepithelial NH$_4^+$ absorption. By contrast, in the rabbit TAL, furosemide inhibited only ~75% of active transcellular NH$_4^+$ flux (Garvin, Burg, and Knepper, 1985), indicating a role for a furosemide-independent apical NH$_4^+$ transport pathway. The apparent absence of furosemide-insensitive transepithelial NH$_4^+$
transport in the rat TAL may be due to species differences. Alternatively, the effects of paracellular and transcellular ammonium fluxes, as well as backfluxes from basolateral to apical solutions, may have obscured furosemide-insensitive NH$_4^+$ transport in studies of net transepithelial NH$_4^+$ transport. Thus, all species tested to date absorb NH$_4^+$ via the apical Na$^+$:K$^+$:NH$_4^+$:2Cl$^-$ cotransporter, yet there may be considerable species differences in the role of the apical, barium-sensitive K$^+$(NH$_4^+$) pathway in NH$_4^+$ absorption (mouse > rabbit > rat).

The Ba$^{2+}$-sensitive NH$_4^+$ transport in MTAL: pH sensitivity and permeation via the apical K$^+$ channel. Fig. 14A summarizes the effects of pH$_i$ on the apical Ba$^{2+}$-sensitive transcellular conductance ($G_c$; solid line) in IP-MTAL, the apical, Ba$^{2+}$-sensitive NH$_4^+$ transport pathway in IP-MTAL (dotted line), and the Ba$^{2+}$-sensitive NH$_4^+$ transport pathway in S-MTAL (dashed line; see below). In both S-MTAL and IP-MTAL preparations, $J_{NH_4}$ was quite sensitive to pH$_i$ changes over the physiological pH range 6–8. The finding that the pH$_i$/$J_{NH_4}$ relationships obtained in IP-MTAL were similar (Fig. 8; dotted line in Fig. 14A) whether pH$_i$ was altered by basolateral pH$_o$, or by luminal amiloride at constant apical and basolateral pH$_o$, indicated that the pH sensitivity of this Ba$^{2+}$-sensitive NH$_4^+$ transport pathway in IP-MTAL is cytosolic rather than extracellular.
The similarities of the effects of pH on Gc and the apical, Ba2+-sensitive NH4+ influx suggests that both K+ and NH4+ are being transported via the same or strikingly similar conductive pathways in apical membranes of MTAL cells. This possibility is supported by several recent observations. A Ba2+-inhibitable K+ channel with similar cytosolic pH sensitivity was observed by Bleich et al. (1990) in recent patch clamp studies of the apical membrane from in vitro perfused rat TAL segments. This was the only type of K+ channel identified in apical membranes of rat TAL by these investigators, and the other observed properties of this channel indicated that it belonged to the class of ATP-regulated, inwardly rectifying K+ channels (KATP). In fact, KATP channels with similar pH sensitivities have also been identified in patch clamp studies of principal cells from the rat renal cortical collecting duct (Wang et al., 1990), of early distal tubule cells from the kidney of Rana pipens (Hunter, Oberleithner, Henderson, and Giebisch, 1988; Wang, Henderson, Geibel, White, and Giebisch, 1989), and of B cells from the pancreatic islet (Rosario and Rojas, 1986a; Misler, Gillis, and Tabcharanii, 1989). While Bleich et al. (1990) found that the KATP channel in apical membranes of rat TAL had similar permeabilities for K+ and NH4+, they were unable to demonstrate any significant NH4+ current in cell excised patches (although this issue was not extensively evaluated in this study). Interestingly, the KATP channel found in pancreatic B cells does appear to exhibit a significant NH4+ permeability, the permeability ratio \( P_{K^+}/P_{NH4^+} \) estimated from fitting the I-V relations to the Goldman-Hodgkin-Katz equation, was 1:3 (Rosario and Rojas, 1986b). Finally, recent studies using site-directed mutagenesis of the Drosophila Shaker K+ channel (although not a KATP-type channel) has demonstrated that certain mutations involving the H5 region, thought to line the channel pore (Guy and Conti, 1990; Yellen, Jurman, Abramson, and MacKinnon, 1991), significantly increased single channel NH4+ conductance (Yool and Schwarz, 1991). Thus it is possible that K+ and NH4+ are being transported across apical membranes of the mouse MTAL via the same KATP channels.

From the curves shown in Fig. 14A, it is also evident that the relative reduction in \( J_{NH4} \) observed with decreasing pH in S-MTAL (Fig. 14A, dashed line) was much steeper than that observed in the IP-MTAL (Fig. 14A, solid and dotted lines). One or more of at least three factors may have accounted for the rightward shift of the \( J_{NH4} \)-pH curve in S-MTAL. First, because basolateral membranes of MTAL cells are highly permeable to NH3, \([NH3]_i = [NH3]_o\) in the presence of basolateral NH4+/NH3, and consequently, \([NH4^+]_i\) will increase with decreasing pH. pH-dependent increases in \([NH4^+]_i\) would in turn reduce the electrochemical gradient for NH4+ entry, and thus, contribute to the pH-associated reduction of \( J_{NH4} \) in S-MTAL. In contrast, \([NH4^+]_i\) would not increase appreciably in IP-MTAL since basolateral medium did not contain NH4+. A second possible explanation for the rightward shift of the \( J_{NH4} \)-pH curve in S-MTAL is that intracellular NH4+/NH3 may have affected the activity of the Ba2+-sensitive NH4+ transport pathway, independent of its effects on the NH4+ chemical gradient. While we know of no specific data addressing this issue for the KATP channel, a regulatory role for ammonium on another K+(NH4+) transporter has been suggested by other investigators (Kurtz and Balaban, 1986; Hamm, Gillespie, and Klahr, 1985). A third possible reason for the different pH sensitivities of the Ba2+-sensitive NH4+ transport pathway in S-MTAL and IP-MTAL may be
related to differences in cytosolic ATP (or other as yet unknown cytosolic factors regulating $K_{ATP}$ channels). In support of this possibility, Misler et al. (1989) found that the $pHi$ sensitivity of the $K_{ATP}$ channel observed in cell-attached membrane patches of pancreatic B cells was essentially abolished in cell-detached, inside-out patches. Furthermore, exposure of the inside-out patches to small concentrations of ATP restored much of the $pHi$ sensitivity of these channels.

$pH_i$ regulation of $Na^+:K^+(NH_4^+):2Cl^-$ cotransport. The apical $Na^+:K^+(NH_4^+):2Cl^-$ cotransporter mediated high rates of $NH_4^+$ entry into MTAL cells, in agreement with previous observations in TAL cells (Kinne et al., 1986; Garvin, Burg, and Knepper, 1988). $NH_4^+$ influx via $Na^+:K^+(NH_4^+):2Cl^-$ cotransport was sensitive to $pHi$ (Fig. 13). Paris and Pouysségur (1986) have shown previously that the activity of growth factor-activated $Na^+:K^+:2Cl^-$ cotransport in fibroblasts was also reduced by cell acidification from ~7.5 to 6.5 pH units. Our observations are qualitatively similar. The mechanisms by which changes in $pHi$ alter $Ba^{2+}$-sensitive $NH_4^+$ transport and $Na^+:K^+(NH_4^+):2Cl^-$ cotransport are unknown.

### Table 1

| Buffer  | $pHi$ | $V_e$ | $G_e$ | $J_e$ |
|---------|-------|-------|-------|-------|
| HEPES   | 7.41  | 8.9   | 123.4 | 10,800 |
| CO$_2$/HCO$_3^-$ | 7.23 | 6.5   | 119.5 | 7,900  |

$J_e = \text{rate of net NaCl absorption calculated as } (V_e \cdot G_e)/F$.

*Data from Kikeri et al., 1990a.

1 Data from Hebert, 1987.

Fig. 14 B compares the $pHi$ sensitivity curves of the apical, $Ba^{2+}$-sensitive $NH_4^+$ transport pathway and the apical, furosemide-sensitive $Na^+:K^+(NH_4^+):2Cl^-$ cotransporter observed in the present IP-MAL studies. In the absence of basolateral/ intracellular $NH_4^+/NH_3$, the $pHi$ sensitivity curve of $Na^+:K^+(NH_4^+):2Cl^-$ cotransport is located ~0.5 pH units to the right of the $pHi$ sensitivity curve of the apical $Ba^{2+}$-sensitive $NH_4^+$ transport pathway.

The differences in the $pHi$ sensitivities of the $Na^+:K^+(NH_4^+):2Cl^-$ cotransporter and $K^+(NH_4^+)$ channel observed in this study can explain our prior observation that switching from HEPES- to (CO$_2$/HCO$_3^-$)-buffered media diminished salt absorption in the mouse IP-MTAL (Hebert, 1987; Kikeri et al., 1990a). The pertinent results from these studies are summarized in Table I. Addition of CO$_2$/HCO$_3^-$ to the external solutions bathing the IP-MAL resulted in a reduction in $V_e$ from 7.41 to 7.23. This cell acidification was associated with 17% decreases in $V_e$ and the rate of NaCl absorption ($J_e$), but no significant change in $G_e$. These results are entirely consistent with the differences in the $pHi$ sensitivities of the two apical $NH_4^+$ transporters shown in Fig. 14 B. The arguments are as follows. If the (CO$_2$/HCO$_3^-$)-mediated fall in $pHi$ altered the apical cotransporter and not the apical $K^+(NH_4^+)$ channel, then the fall in
NaCl absorption should be quantitatively predicted by the pH$_i$ sensitivity of the cotransporter. Using the pH$_i$ titration equation fitted to the data in Figs. 13 and 14 B, the predicted rates of cotransporter activity would be 14.3 mM/min at pH$_i$ 7.41 and 11.0 mM/min at pH$_i$ 7.23, or a fall in cotransporter activity of ~13%, a value quite similar to the observed 17% fall in $J_e$. On the other hand, the lack of a significant change in $G_e$ (Table I) is consistent with the lower pH$_i$ sensitivity of the K$^+$(NH$_4^+$) channel (Fig. 14 B).

Model of Effects of pH$_i$ on Transcellular NH$_4^+$ Transport in MTAL

A model for the regulation of NH$_4^+$ transport, based on these and previous observations (Kikeri et al., 1989, 1990a), is presented in Fig. 15. Fig. 15 A shows the steady-state concentrations of extracellular and intracellular NH$_4^+$ and NH$_3$ in MTAL cells in the presence of 5 mM ambient NH$_4$Cl (luminal/basolateral medium pH 7.4). Although apical membranes of mouse MTAL cells are virtually impermeable to NH$_3$, the concentrations of intracellular and basolateral (interstitial) NH$_3$ would be virtually equal since basolateral membranes of MTAL cells are highly permeable to NH$_3$ (Kikeri et al., 1989). Thus, the concentration of intracellular NH$_4^+$ will depend on the pH$_i$ and the NH$_3$ concentration of the basolateral medium. For this example the steady-state pH$_i$ in the presence of 5 mM ambient NH$_4$Cl would be 7.13. Fig. 15 B depicts the effects of both NH$_4^+$-induced pH$_i$ changes and intracellular NH$_4^+/NH_3$ on transcellular NH$_4^+$ transport in the mouse MTAL in the presence of ambient 5 mM NH$_4$Cl. NH$_4^+$ entry from the lumen would result in the net generation of H$^+$ with NH$_3$ diffusing down its gradient into the medullary interstitium. Because of the negligible apical membrane NH$_4$ permeability, NH$_3$ backleak from the cytoplasm to the lumen would be minimal (Kikeri et al., 1989). Because of the large cellular buffering power, $B_n$, most of the H$^+$ load due to NH$_4^+$ entry would be buffered, thus attenuating the drop in pH$_i$. The increasing $B_n$ with acidification below a pH of 7.0 (Fig. 2) would also help to attenuate pH$_i$ changes resulting from apical NH$_4^+$ entry. Nevertheless, cell acidification due to NH$_4^+$ entry would (a) inhibit NH$_4^+$ entry via the apical entry pathways, and (b) increase the rate of apical (Kikeri et al., 1990a) and basolateral (Sun and Hebert, 1990) Na$^+$/H$^+$ exchange. The combined effect of reduced NH$_4^+$ entry and increased Na$^+$/H$^+$ exchange would result in an increase in pH$_i$, which would tend to restore NH$_4^+$ entry via the apical entry pathways and decrease the rate of Na$^+$/H$^+$ exchange. At the steady-state pH$_i$ in the presence of 5 mM ambient NH$_4$Cl (7.13 pH units), H$^+$ influx due to NH$_4^+$ entry would be balanced by H$^+$ efflux (predominantly via Na$^+$/H$^+$ exchange) due to NH$_4^+$ exit (Fig. 5).

Some Physiological Implications of This Model for NH$_4^+$ and NaCl Transport

Changes in pH$_i$ in the presence of basolateral/intracellular NH$_4^+/NH_3$ may affect the rate of apical NH$_4^+$ transport by altering the NH$_4^+$ chemical gradient. Thus, the concentration of NH$_3$ in the medullary interstitium surrounding the MTAL may play an important role in regulating transcellular NH$_4^+$ absorption by the in vivo MTAL; the rate of transcellular NH$_4^+$ flux would be high in the presence of low interstitial NH$_3$ concentrations and vice versa. In other words, as the medullary interstitial concentration of NH$_4^+/NH_3$ rises, transcellular NH$_4^+$ transport would be inhibited because of an increase in intracellular NH$_4^+/NH_3$ concentrations. The inhibitory effect
FIGURE 15. (A) Steady-state intracellular and extracellular concentrations of NH$_4^+$/NH$_3$ in the nominal presence of 5 mM NH$_4$Cl in both apical and basolateral media (pH$_i$ 7.40) at pH$_i$ 7.13. The pKa for ammonium was 9.0. (B) Model of role of pH$_i$ and NH$_4^+$/NH$_3$ in regulating transcellular NH$_4^+$ transport in the mouse MTAL. The single arrow showing apical NH$_4^+$ entry represents NH$_4^+$ entry via both the apical, Ba$^{2+}$-sensitive NH$_4^+$ pathway and the apical, Na$^+$.K$^+$/NH$_4^+$:2Cl$^-$ pathway. See text for detailed discussion.
of intracellular \( \text{NH}_3/\text{NH}_4 \) on transcellular \( \text{NH}_4 \) transport could be due to either a reduction in the \( \text{NH}_4 \) chemical gradient or an effect (either directly or indirectly) of intracellular \( \text{NH}_3/\text{NH}_4 \) on the apical \( \text{NH}_4 \) transport pathways. This "negative feedback loop" would limit the maximum interstitial concentrations of \( \text{NH}_4/\text{NH}_3 \), which in turn is believed to play an important role in regulating renal \( \text{NH}_4 \) excretion (Knepper et al., 1989).

\( \text{NaCl} \) reabsorption by the MTAL both dilutes the urine and provides the single effect of the countercurrent multiplication process which is required for vasopressin-dependent concentration of urine in the medullary collecting duct. Net \( \text{NaCl} \) absorption by the mouse MTAL is dependent on the activities of both the apical \( \text{Na}^+\text{-K}^+\text{-2Cl}^- \) cotransporter and the apical \( \text{K}^+ \) channel (Hebert and Andreoli, 1986). Given the results of this study, it seems reasonable to speculate that the pH, and/or the presence of basolateral/intracellular \( \text{NH}_3/\text{NH}_4 \) may affect the rate of transepithelial \( \text{NaCl} \) absorption in the mouse MTAL by altering the activities of both of these ion transporters. Several lines of evidence support this possibility. First, as discussed above, the results in Table I are consistent with this notion. Second, Wingo (1986) has demonstrated that both respiratory and metabolic acidosis result in a reduction of transepithelial \( \text{Cl}^- \) transport in the TAL. Since both an increase in ambient CO\(_2\) concentration (Kikeri et al., 1990\(a\)) and metabolic acidosis would be expected to lead to cell acidification, inhibition of apical ion transport pathways in the TAL by cell acidification may explain the acidosis-induced reduction of transepithelial \( \text{Cl}^- \) absorption observed by Wingo (1986). Third, the MTAL in the isolated perfused rat kidney is exquisitely vulnerable to hypoxic injury (Brezis, Rosen, Silva, and Epstein, 1984\(a\)) because of the high rates of transport-related energy consumption by the MTAL (measured as ouabain-sensitive oxygen consumption [Brezis, Rosen, Silva, and Epstein, 1984\(b\)]) due to the high rates of transport-related energy consumption by the MTAL (measured as ouabain-sensitive oxygen consumption [Brezis, Rosen, Silva, and Epstein, 1984\(b\)]) due to the high rates of transport-related energy consumption by the MTAL (measured as ouabain-sensitive oxygen consumption [Brezis, Rosen, Silva, and Epstein, 1984\(b\)]) due to the high rates of transport-related energy consumption by the MTAL (measured as ouabain-sensitive oxygen consumption [Brezis, Rosen, Silva, and Epstein, 1984\(b\)].) Reduction of the perfusate pH (acidosis) markedly attenuates hypoxic injury to MTAL in the isolated perfused rat kidney (Shanley, Shapiro, Chan, Burke, and Johnson, 1988). An attractive explanation for the acidosis-induced protection against hypoxic MTAL cell injury is that inhibition of ion transport-dependent oxygen consumption in the MTAL (> 50% of total oxygen consumption [Kikeri et al., 1990\(a\)]) by cell acidification may protect against cell damage in hypoxic conditions. Fourth, it has long been recognized that the oral administration of an \( \text{NH}_4\text{Cl} \) load leads to diuresis, natriuresis, and kaliuresis without a consistent change in glomerular filtration rate (Pitts, 1959; Sartorius, Roemmelt, and Pitts, 1949). Inhibition of apical ion transporters in the TAL and possibly in other nephron segments by \( \text{NH}_4 \)-induced cell acidification or by intracellular \( \text{NH}_3/\text{NH}_4 \) itself may at least partially explain the effects of acute \( \text{NH}_4\text{Cl} \) loading on salt and \( \text{H}_2\text{O} \) excretion by the kidney. Finally, alterations in pH, have been suggested to alter transport processes in diluting segments. Weigt, Dietl, Silbernagl, and Oberleithner (1987) and Wang et al. (1989) have suggested that the effect of aldosterone on the apical \( \text{K}^+ \) channel in the frog diluting segment is mediated by cell alkalinization due to activation of \( \text{Na}^+\text{-H}^+ \) exchange.

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