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**Research Article**

The epithelia of the medullary thick ascending limb (MAL) consists of two cell types, high (HBC) and low basolateral conductance (LBC) cell, depending on the K+ conductance of the basolateral membrane. The NH4+ conductance distinct from the K+ conductance has been suggested to exist in the proximal tubule, MAL cell, and Xenopus oocyte. The present study was designed to examine whether there is a conductive NH4+ transport system distinct from K+ conductance in two different cell types of the hamster MAL perfused in vitro. The basolateral membrane voltage (VB) was measured by impaling cells with conventional microelectrodes. Addition of NH4+ to the bath depolarized VB in a dose-dependent manner in both cell types. The response was maintained in the absence of HCO3-. When the VB deflection elicited upon 50 mM KCl or NH4Cl in the bath (delta VBK+ or delta VBNH4+) were compared, delta VBNH4+ was almost the same as delta VBK+ in the HBC cell, whereas the former was greater than the latter in the LBC. In the HBC cell, 10 mM Ba2+ in the bath equally suppressed both delta VBK+ and delta VBNH4+, whereas in the LBC cell it suppressed delta VBK+ with a small effect on delta VBNH4+, indicating that NH4+ is transported via a pathway distinct from Ba(2+)-sensitive K+ conductance. The VB deflection by NH4+ was […]



# Cellular Heterogeneity of Ammonium Ion Transport across the Basolateral Membrane of the Hamster Medullary Thick Ascending Limb of Henle's Loop

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## Abstract

**The epithelia of the medullary thick ascending limb (MAL) consists of two cell types, high (HBC) and low basolateral conductance (LBC) cell, depending on the $K^+$ conductance of the basolateral membrane. The $NH_4^+$ conductance distinct from the $K^+$ conductance has been suggested to exist in the proximal tubule, MAL cell, and *Xenopus* oocyte. The present study was designed to examine whether there is a conductive $NH_4^+$ transport system distinct from $K^+$ conductance in two different cell types of the hamster MAL perfused in vitro. The basolateral membrane voltage ($V_B$) was measured by impaling cells with conventional microelectrodes. Addition of $NH_4^+$ to the bath depolarized $V_B$ in a dose-dependent manner in both cell types. The response was maintained in the absence of $HCO_3^-$. When the $V_B$ deflection elicited upon 50 mM KCl or $NH_4Cl$ in the bath ($\Delta V_{B_{K^+}}$ or $\Delta V_{B_{NH_4^+}}$) were compared, $\Delta V_{B_{NH_4^+}}$ was almost the same as $\Delta V_{B_{K^+}}$ in the HBC cell, whereas the former was greater than the latter in the LBC. In the HBC cell, 10 mM $Ba^{2+}$ in the bath equally suppressed both $\Delta V_{B_{K^+}}$ and $\Delta V_{B_{NH_4^+}}$, whereas in the LBC cell it suppressed $\Delta V_{B_{K^+}}$ with a small effect on $\Delta V_{B_{NH_4^+}}$, indicating that $NH_4^+$ is transported via a pathway distinct from $Ba^{2+}$-sensitive $K^+$ conductance. The $V_B$ deflection by $NH_4^+$ was unaffected by addition of 0.1 mM ouabain or 10 $\mu$M 5-nitro-2-(3-phenylpropylamino)-benzoate (a $Cl^-$ channel blocker) to the bath, excluding the contribution of the $Na^+$, $K^+$ pump or $Cl^-$ channel. An abrupt reduction of $Na^+$ in the bath from 200 to 20 mM did not cause any changes in $V_B$, suggesting that a nonselective cation channel may not account for the $NH_4^+$ transport. Amiloride at 10 $\mu$M inhibited $\Delta V_{B_{NH_4^+}}$ with a higher efficacy in the LBC cell. We conclude that a rheogenic $NH_4^+$ transport system independent from the $K^+$ conductance exists in the basolateral membrane of the LBC cell of the hamster MAL, and may play some roles in the regulation of $NH_4^+$ transport. (*J. Clin. Invest.* 1993. 92:1881–1888.) Key words: ammonium transport • $Ba^{2+}$-sensitive $K^+$ channel • Henle's loop • $K^+$ conductance • $NH_4^+$ conductance**

## Introduction

Ammonium transport in the kidney plays an important role in urinary acid excretion (1, 2). Good et al. (3) demonstrated for the first time that, in the thick ascending limb of Henle's loop, the ammonium ion ($NH_4^+$) is directly transported as opposed to the nonionic diffusion of $NH_3$. Subsequent studies by Garbin et al. (4) and Good (5) suggested that the greatest fraction of $NH_4^+$ absorbed in this segment is mediated by a secondary active process through a $Na^+$-$NH_4^+$-$2Cl^-$ cotransport across the apical membrane.

Several lines of evidence supported the view that $NH_4^+$ substitutes for $K^+$ on $Na^+$-$K^+$-$2Cl^-$ cotransporter in the apical membrane. First, Kinne et al. (6) demonstrated in apical membrane vesicles from rabbit thick ascending limbs that the $NH_4^+$ gradient, as well as the $K^+$ gradient, can drive bumetanide-sensitive $^{22}Na$ uptake. Second, Good (5) demonstrated in the rat thick ascending limb that $K^+$ in the lumen competitively inhibits $NH_4^+$ absorption. Third, Garvin et al. (4) reported that furosemide inhibits the active $NH_4^+$ absorption independent of its effect on the transmural voltage. Fourth, they further showed that the complete replacement of $K^+$ by $NH_4^+$ maintains the active $Cl^-$ transport across the thick ascending limb. Finally, Kikeri et al. (7) demonstrated that, in the mouse thick ascending limb, the luminal addition of $NH_4^+$ causes a marked decrease in intracellular pH, indicating that $NH_4^+$ rather than $NH_3$ preferentially crosses the apical membrane.

The major route for net ammonia absorption across the thick ascending limb thus established is as follows: $NH_4^+$ enters cell across the apical membrane and dissociates in the cell to proton and $NH_3$, the latter of which exits across the basolateral membrane by simple passive diffusion. In contrast, the possible existence of other routes for $NH_4^+$ transport across cell membranes of the thick ascending limb is less well known. Although it is possible that $NH_4^+$ is also transported via $K^+$ conductance in the apical membrane, the results reported by several groups of investigators are controversial. Garvin et al. (4) reported that, in rat thick ascending limb, there was net $NH_4^+$ flux which was not inhibited by furosemide. Kikeri et al. (7) reported that, in mouse thick ascending limb, barium partially blocked the entry of $NH_4^+$ from the apical membrane. However, Kinne et al. (6) reported that bumetanide-insensitive $^{86}Rb$ uptake in apical membrane vesicles of the rabbit thick ascending limb was not affected by $NH_4^+$. Furthermore, a patch clamp study on the apical membrane of the thick ascending limb showed that the $K^+$ channel is less conductive to $NH_4^+$ and that $NH_4^+$ rather inhibits $K^+$ current (8).

Yoshitomi et al. (9) reported that, in the hamster medullary thick ascending limb of Henle's loop (MAL),[1] there are two cell types: one having a high basolateral membrane con-

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1. *Abbreviations used in this paper:* HBC, high basolateral membrane conductance; LBC, low basolateral membrane conductance; MAL, medullary thick ascending limb; NPPB, 5-nitro-2-(3-phenylpropylamino)-benzoate; $V_B$, basolateral membrane voltage; $\Delta V_{B_{K^+}}$, voltage deflection of basolateral membrane induced by $50K^+$ solution in the bath; $\Delta V_{B_{NH_4^+}}$, voltage deflection of basolateral membrane induced by $50NH_4^+$ solution in the bath.

ductance (HBC) and the other having a low basolateral membrane conductance (LBC). They are comparable to those reported for the early distal tubule of the *Amphiuma* kidney (10). Distinguishing between these cell types is critically dependent on the difference in the magnitude of basolateral membrane $K^+$ conductance. Although $NH_4^+$ is known to share various transport mechanisms with $K^+$ as mentioned above, lines of evidence have accumulated in support of the view that there is an $NH_4^+$ conductance which is distinct from the $K^+$ conductance. Völkl and Lang (11) found that, in the mouse straight proximal tubule, a rheogenic $NH_4^+$ entry mechanism exists in the basolateral membrane. Bichara et al. (12) made a preliminary report on the existence of $NH_4^+$ conductance in the rat MAL cell preparation through measuring intracellular pH and voltage by fluorometry. Burckhardt and Frömter (13) also found $NH_4^+$ conductance distinct from the $K^+$ conductance in *Xenopus* oocyte. Independent of these studies, we have also noticed while we were studying paracellular conductance of $NH_4^+$ in the segments of Henle's loop that increases in $NH_4^+$ concentration in the bath caused marked depolarization of the basolateral membrane voltage. Therefore, the present study was designed to examine whether there is a conductive $NH_4^+$ transport system distinct from the $K^+$ conductance in two different cell types of the hamster MAL perfused in vitro. In this study we found that there is remarkable cell heterogeneity with regard to the basolateral membrane $NH_4^+$ conductance which is distinct from the $K^+$ conductance.

## Methods

*In vitro microperfusion.* Male or female golden hamsters weighing 60–110 g were maintained on regular laboratory diet and allowed free access to tap water *ad libitum*. On the days of experiments, the animals were decapitated with a guillotine. Both kidneys were removed and placed in a dish containing modified Collins solution of the following composition (mM); 14 $KH_2PO_4$, 44 $K_2HPO_4$, 15 KCl, 9 $NaHCO_3$, and 360 sucrose (pH 7.4), maintained at 4–5°C. The cortical portion was removed by fine forceps and the remaining block of renal medulla was transferred to another dish, which contained the same solution. Segments of the MAL were isolated with fine forceps under a stereomicroscope. Isolated renal tubules were transferred to a perfusion bath mounted on an inverted microscope (IMT 2-21, Olympus, Tokyo) and perfused in vitro at 37°C according to the method of Burg et al. (14), as modified previously (9). A system of a flow-through bath was utilized to permit rapid exchange of the bathing fluid. The bathing fluid was maintained at 37°C by supplying through a warm water jacket. The flow rate of the bathing fluid was ranged from 3 to 5 ml/min. The compositions of solutions used in this study are listed in Table I.

*Electrophysiological studies.* Transmural voltage ($V_T$) was measured by connecting a 1 M KCl agar bridge to a saturated KCl reservoir where a calomel half-cell electrode was placed. The electrode was connected to a dual channel electrometer (Duo 773, WP Instruments, New Haven, CT) and recorded on a two-pen recorder (R-301, Rikadenki, Tokyo). The circuit was completed by connecting to another calomel half-cell electrode, which was connected to the bath with a 1 M KCl agar bridge, serving as a common ground.

Basolateral membrane voltage was measured by intracellular impalement of the epithelia of perfused segment with a conventional microelectrode fabricated by a vertical puller (PE-2, Narishige, Tokyo). Electrodes were filled with 0.5 M KCl and connected to another channel of the electrometer via a holder which contains Ag-AgCl pellet. The position of electrodes was controlled with manipulators (MO-102M, Narishige) fixed on the stage of the inverted microscope. Impalement of an electrode was conducted by table tapping or current oscillation.

*Solutions and chemicals.* The composition of the solutions used in this study is listed in Table I. Solutions containing $HCO_3^-$ were bubbled with 95% $O_2$ and 5% $CO_2$ to adjust pH at 7.4. $HCO_3^-$-free solutions were bubbled with 100% $O_2$. The pH of those solutions was adjusted to 7.4 by Hepes and Tris. 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB) was kindly supplied by Hoechst (Frankfurt, FRG). Ouabain and amiloride were purchased from Sigma Chemical Co. (St. Louis, MO).

*Data analysis.* The initial peak of the change in the basolateral membrane ($\Delta V_B$) after rapid exchange of the bathing fluid was taken as a value reflecting an apparent conductance of the ion in question. When $\Delta V_B$ induced by adding 50 mM KCl to the bath was more than 20 mV, the cell was regarded to be HBC cell and others were regarded to be LBC cell.

All data are expressed as means±SE. Statistical analysis was performed by using the Student's *t* test for paired or unpaired samples when appropriate. *P* values $< 0.05$ were considered as significant.

## Results

*$V_B$ deflection upon abrupt changes in $K^+$ or $NH_4^+$ concentration in the bath.* Impalement of MAL cells with an conventional microelectrode revealed negative basolateral membrane voltage of ~ 70–80 mV, which was stabilized within a few minutes. Initially, we examined effects of abrupt change in $NH_4^+$ concentration in the bathing fluid on $V_B$ in the solutions containing 25 mM $HCO_3^-$ bubbled with 95% $O_2$/5% $CO_2$. To discriminate between two types of cell, HBC and LBC cells, $K^+$ concentration in the bath was increased from 5 to 50 mM. In

*Table I. Composition of Solutions Used in This Study*

| | Control (A) | 50$K^+$ (A) | 50$NH_4^+$ (A) | 0$HCO_3^-$ (B) | 50$K^+$ (B) | 50$NH_4^+$ (B) | 0$Na^+$ | 0$Na^+$-50$K^+$ | 0$Na^+$-50$NH_4^+$ |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | *mM* | | | | |
| NaCl | 200 | 155 | 150 | 215 | 170 | 165 | 0 | 0 | 0 |
| Choline Cl | 0 | 0 | 0 | 0 | 0 | 0 | 210 | 164 | 160 |
| $NaHCO_3$ | 25 | 25 | 25 | 0 | 0 | 0 | 0 | 0 | 0 |
| KCl | 5 | 50 | 5 | 5 | 50 | 5 | 5 | 50 | 5 |
| $NH_4Cl$ | 0 | 0 | 50 | 0 | 0 | 50 | 0 | 0 | 50 |
| Others | * | * | ‡ | *‡ | *‡ | *‡ | ‡§ | ‡§ | ‡§ |

All solutions contained 8.3 D-glucose, 5 L-alanine, and 100 urea. When $NH_4^+$ concentration was varied, NaCl was replaced by $NH_4Cl$. * (mM) = 1$MgCl_2$ + 1.8$CaCl_2$ + 0.8$Na_2HPO_4$ + 0.2$NaH_2PO_4$ + 10Na acetate. ‡ (mM) = 10Hepes + 5Tris. § (mM) = 1Mg acetate + 1.8 Ca acetate + 1$KH_2PO_4$.

support of the observation of Yoshitomi et al. (9), we confirmed that there are two types of cell depending on the magnitude of the voltage deflection of the basolateral membrane in response to $K^+$ concentration challenge. After discriminating two cell types, the bathing fluid was changed to solutions of which NaCl was replaced with various concentrations of $NH_4Cl$. Representative tracings are shown in Fig. 1. Fig. 1 *a* represents a tracing of a HBC cell; Fig. 1 *b* shows a LBC cell. In both cells, replacement of $Na^+$ with $NH_4^+$ caused a sharp positive deflection of $V_B$ ($\Delta V_B$) in a dose-dependent manner. The results of 14 experiments are summarized in Fig. 1 *c*. It is noteworthy that the dose-response curve in LBC cell is almost identical to that in HBC cell in spite of the marked difference in the $V_B$ response to 50 mM $K^+$ challenge.

*Effect of $HCO_3^-$ on $V_B$ response to $K^+$ and $NH_4^+$.* Although the $HCO_3^-$-containing ambient solutions are physiological, the interpretation on the effect of $NH_4^+$ may be complicated under such conditions. It is possible that the alkalinization of the cell by diffusion of $NH_3$ may accelerate the extrusion of $HCO_3^-$ via a rheogenic $Na^+$-$3HCO_3^-$ cotransport which is supposed to exist in the basolateral membrane of the thick ascending limb (15, 16), leading to depolarization of the basolateral membrane. To estimate the contribution of this component, we examined the effect of $NH_4^+$ or $K^+$ in the bath in the presence or absence of $HCO_3^-$ in ambient solutions. A representative tracing of the $V_B$ of a LBC cell under this protocol is shown in Fig. 2. While the tubule was perfused with A solution, abrupt changes in $K^+$ or $NH_4^+$ in the bathing fluid were conducted to observe changes in $V_B$ of the cell with impalement of an electrode. From the responses to $50K^+$ and $50NH_4^+$, the cell was identified as a LBC cell. When the bathing fluid was changed to bicarbonate free B solution, the $V_B$ depolarized by 9 mV. Under this condition, the $V_B$ responses to $50K^+$ and $50NH_4^+$ were unchanged. The results of this protocol are summarized in Table II. In both HBC and LBC cells, elimination of bicarbonate from the ambient fluid caused significant depolarization of the basolateral membrane. However, the $V_B$ responses to $50K^+$ and to $50NH_4^+$ were unchanged in the absence of bicarbonate. The following studies were conducted in the absence of bicarbonate in ambient solutions.

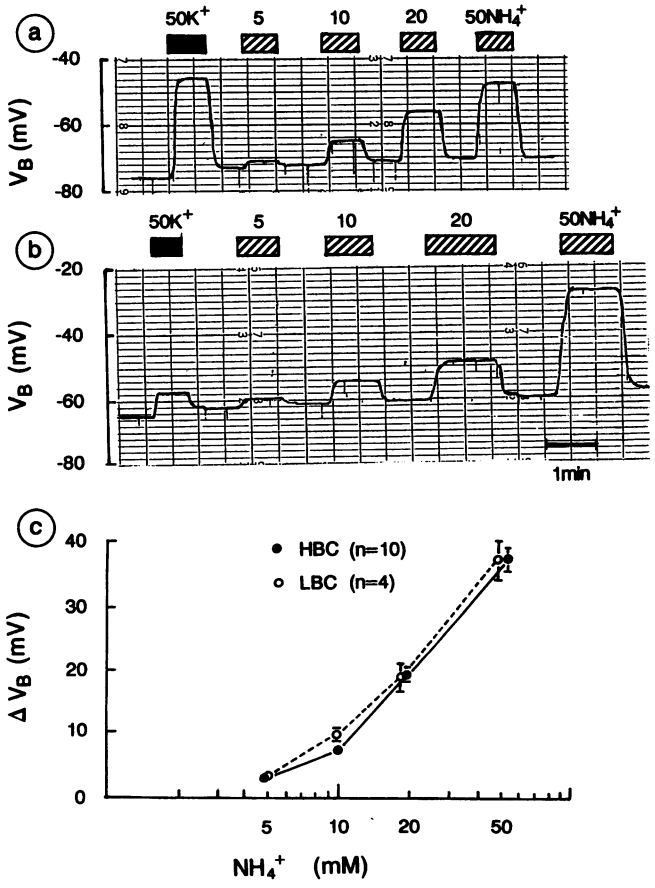


*Figure 1.* Deflection of basolateral membrane voltage ($V_B$) upon abrupt changes in concentration of $K^+$ or $NH_4^+$ in the bath. (*a* and *b*) Representative tracings of $V_B$ of HBC cell and LBC cell, respectively. Note that in spite of big difference of $V_B$ response to $50K^+$ between two cells, the magnitude of the response to $NH_4^+$ are very similar. (*c*) Summaries of concentration-dependent $V_B$ responses to $NH_4^+$ are shown.

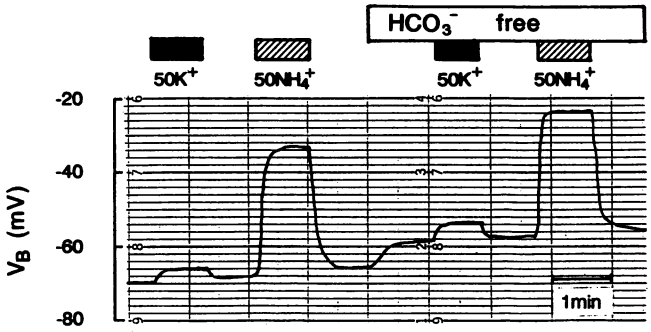


*Figure 2.* A representative tracing of $V_B$ responses to $50K^+$ or $50NH_4^+$ in the bath in the presence or absence of $HCO_3^-$. The cell is identified as LBC cell based on the small response to $50K^+$.

*Dissociation of $V_B$ responses to 50 $K^+$ and 50 $NH_4^+$ challenge.* The $V_B$ response to a rapid change in ion concentration in the bath is a measure of apparent ion conductance of the basolateral membrane. By random impalement of perfused

*Table II. Effect of Elimination of $HCO_3^-$ on Voltage Responses of the Basolateral Membrane to $50K^+$ or $50NH_4^+$ Challenge in the Bath in the Hamster MAL*

| | $HCO_3^-$ | HBC cell | LBC cell |
|---|---|---|---|
| | *mM* | | |
| *n* | | 4 | 4 |
| $V_B$ | C 25 | −80.6±2.8 | −65.0±1.3 |
| | E 0 | −68.4±6.3 | −56.5±2.4 |
| | E-C | 12.2±3.1* | 8.5±1.8‡ |
| $\Delta V_B(50K^+)$ | C 25 | 47.0±1.7 | −5.8±1.2 |
| | E 0 | 46.0±1.2 | −7.3±1.0 |
| | E-C | −1.0±0.6 | −1.5±0.5 |
| $\Delta V_B(50NH_4)$ | C 25 | 48.5±2.3 | 42.8±3.3 |
| | E 0 | 45.3±2.4 | 40.8±2.4 |
| | E-C | −3.3±3.7 | −2.0±1.3 |

Abbreviations: $V_B$, basolateral membrane voltage; $\Delta V_B(50K^+)$, voltage deflection caused by $50K^+$ solution in the bath; $\Delta V_B(50NH_4^+)$, voltage deflection caused by $50NH_4^+$ solution in the bath; C, control period; E, experimental period. * $P < 0.05$, ‡ $P < 0.01$ as compared to zero.

MAL with a microelectrode, we compared the magnitude of the voltage deflection caused by $50K^+$ or $50NH_4^+$ solution in the bath. We obtained the data of 101 cells from 66 MAL tubules. The mean $V_B$ in the control solution was −72.6 mV. The distribution of $\Delta V_B$ by abrupt exposure to $50K^+$ bath solution ($\Delta V_{B_{K^+}}$) clearly show that there are two cell populations with respect to the response to the $K^+$ challenge (Fig. 3). The $\Delta V_{B_{K^+}}$ was 40.4±0.8 mV in HBC cells and 7.4±0.7 mV in LBC cells. These two cell types were sometimes observed in the same tubules. The basal levels of $V_B$ were not different between two groups; −73.2±1.0 mV in the HBC group ($n$ = 63) and −71.6±1.1 mV in the LBC group ($n$ = 38). In spite of clear distinction of $\Delta V_{B_{K^+}}$, the $\Delta V_B$ upon 50 mM $NH_4^+$ in the bath ($\Delta V_{B_{NH_4^+}}$) was not different between two groups; 37.0±0.9 mV in the HBC cell and 35.3±0.9 mV in the LBC cell. In the HBC cell, $\Delta V_{B_{K^+}}$ was slightly but significantly higher than $\Delta V_{B_{NH_4^+}}$ (40.4±0.8 vs. 37.0±0.9 mV, $P < 0.01$).

Since the thick ascending limb is a leaky segment (9, 17), it is possible that the observed $\Delta V_{B_{K^+}}$ or $\Delta V_{B_{NH_4^+}}$ may be underestimated by the circular current through the paracellular shunt pathway. To exclude this component, we observed $\Delta V_{B_{K^+}}$ or $\Delta V_{B_{NH_4^+}}$ in nonperfused renal tubules. The data are summarized in Fig. 4, comparing with the data obtained from perfused renal tubules. In both HBC and LBC cells, the $V_B$ responses to $50K^+$ and to $50NH_4^+$ were not different between the perfused and nonperfused tubules. Although the $V_B$ response to $NH_4^+$ was tended to be higher in the nonperfused tubules, the value was not significantly different from that in perfused tubules.

*Effect of barium.* To examine whether $\Delta V_{B_{NH_4^+}}$ represents $NH_4^+$ transport through a $K^+$ conductance, we observed effects of 10 mM $BaCl_2$ in the bath on $\Delta V_{B_{K^+}}$ and $\Delta V_{B_{NH_4^+}}$. Representative tracings for each cell type are shown in Fig. 5. In the HBC cell shown in the upper panel, an addition of 10 mM $Ba^{2+}$ in the bath markedly depolarized the basolateral membrane. In the presence of $Ba^{2+}$, an abrupt increase in the bath $K^+$ concentration to 50 mM further depolarized the basolateral membrane, although the magnitude of the deflection was markedly reduced. This suggests that there are $Ba^{2+}$ insensitive components for $K^+$ conductance. Under the same condition, the challenge with 50 $NH_4^+$ also depolarized the basolateral membrane.

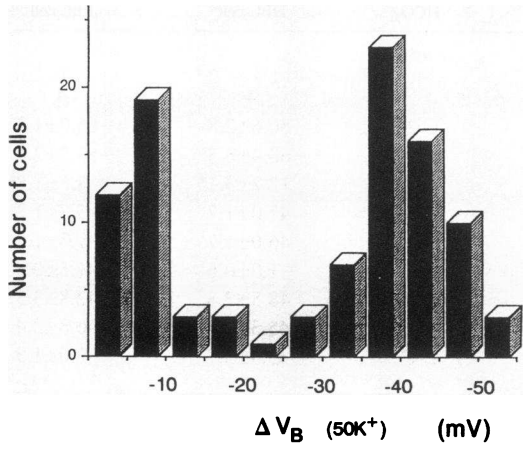


*Figure 3.* Distribution of $V_B$ response to $50K^+$ in the bath in randomly punctured hamster MAL cells. It is evident that there are two cell populations.

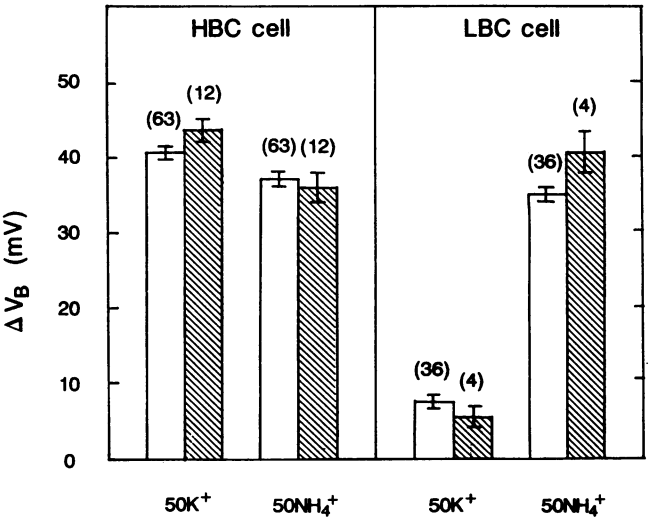


*Figure 4.* Comparison of $V_B$ responses to $50K^+$ or $50NH_4^+$ between HBC cell and LBC cell. Open columns represent the data obtained from perfused tubules, whereas hatched columns the data from nonperfused tubules. Numbers in parentheses indicate number of cells.

In the LBC cell shown in the lower panel, $V_B$ was slightly depolarized by 10 mM $Ba^{2+}$ in the bath. The $V_B$ response to $50K^+$ was almost abolished while the $V_B$ response to 50 mM $NH_4^+$ was retained. The results of this series of experiments are summarized in Fig. 6. $\Delta V_{B_{K^+}}$ was inhibited to almost the same degree in both cells (68.9±2.4% in HBC cells [$n$ = 11] vs. 73.5±6.6% in LBC cells [$n$ = 7]). Although in HBC cells $\Delta V_{B_{NH_4^+}}$ was inhibited by 51.2±3.0%, the inhibition of $\Delta V_{B_{NH_4^+}}$ in LBC cells was much smaller (22.9±1.8%).

*Effect of amiloride.* Because Bichara et al. (12) reported that amiloride at a dose that does not affect $Na^+/H^+$ antiporter (1 μM) inhibited $NH_4^+$ conductance as assessed by pH changes in the suspension of rat medullary thick ascending tubules, we examined effects of amiloride on $\Delta V_{B_{NH_4^+}}$. As shown in representative tracings in Fig. 7, the $V_B$ deflection to $50K^+$ was slightly decreased in both cells and the $V_B$ deflection to $50NH_4^+$ was markedly reduced in the LBC cell, but slightly reduced in the HBC cell. In both cells, small but significant depolarizations of the $V_B$ were noted (HBC, $\Delta V_B$ = 1.4±0.3 mV, $n$ = 11, $P < 0.01$; LBC, $\Delta V_B$ = 0.8±0.2 mV, $n$ = 6, $P < 0.01$) when 10 μM amiloride was added to the bath. These changes, however, are too small to be physiologically significant. In addition, the orientation of the voltage deflection was opposite to that expected from an inhibition of amiloride-sensitive $Na^+$ channel. As summarized in Fig. 8, in the presence of 10 μM amiloride in the bath, $\Delta V_{B_{K^+}}$ was slightly inhibited by 10.9% in HBC cells and by 3.2% in LBC cells. These inhibition rates were not significantly different. The response of $\Delta V_{B_{NH_4^+}}$ was somewhat different. In HBC cells an addition of amiloride inhibited $\Delta V_{B_{NH_4^+}}$ by 22.5±3.1%, whereas in LBC cells by 50.5±6.6%. The inhibition was more prominent in LBC cells ($P < 0.01$). These results suggest that there is an amiloride sensitive $NH_4^+$ conductance predominantly in the LBC cell.

*Effect of NPPB.* To exclude the possibility that $NH_4^+$ loading increases $Cl^-$ conductance of the basolateral membrane due to changes in intracellular pH, we examined $\Delta V_{B_{K^+}}$ or $\Delta V_{B_{NH_4^+}}$ in the presence of 10 μM 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB), a $Cl^-$ channel blocker (18). When

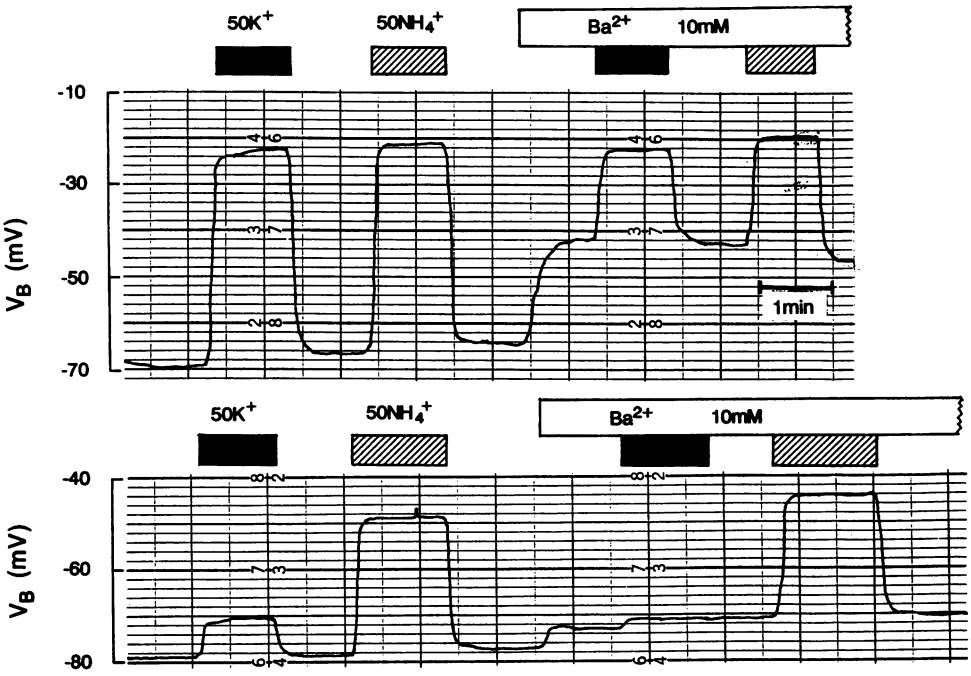


*Figure 5.* Representative tracings showing $V_B$ responses to $50K^+$ or $50NH_4^+$ in the presence or absence of $Ba^{2+}$ in the bath. The upper panel represents the HBC cell, and the lower panel represents the LBC cell.

10 $\mu$M NPPB was added to the bath the $V_B$ of both HBC and LBC cells tended to hyperpolarize from $-75.1\pm4.8$ to $-81.7\pm4.7$ mV ($n = 10$, $P < 0.01$) and from $-68.5\pm3.3$ to $-77.7\pm2.5$ mV ($n = 6$, $P < 0.05$), respectively. As summarized in Table III, the responses of $\Delta V_{B_{K^+}}$ and $\Delta V_{B_{NH_4^+}}$ were not different in the presence or absence of NPPB in both cell types.

*Effect of elimination of $Na^+$.* Because Burckhardt and Frömter (13) suggested that in *Xenopus* oocyte a nonselective cation channel might be responsible for $NH_4^+$ conductance, we tested whether $Na^+$ conductance is detectable in the basolateral membrane of the hamster MAL. When $Na^+$ concentration of the bathing fluid was abruptly reduced from 211.6 to 20 mM, $V_B$ did not change significantly in both cell types (Table IV). Even when $Na^+$ was completely eliminated, $V_B$ did not change significantly (Table IV). The responses of $\Delta V_{B_{K^+}}$ and $\Delta V_{B_{NH_4^+}}$ were also unchanged under reduced $Na^+$ concentration (data not shown).

*Effect of ouabain.* To assess whether the active $Na^+$ transport is required for the response, we examined $\Delta V_{B_{K^+}}$ or $\Delta V_{B_{NH_4^+}}$ in the presence of ouabain. The results are summarized in Table V. In seven HBC cells, 10 $\mu$M ouabain decreased $V_B$ from $-73.0$ to $-48.2$ mV ($P < 0.001$). The responses of $\Delta V_{B_{K^+}}$ in the control and ouabain period were 40.2 and 36.0 mV, respectively ($P > 0.05$). The responses of $\Delta V_{B_{NH_4^+}}$ in comparable periods were 40.2 and 38.0 mV, respectively ($P > 0.05$). In four LBC cells, 10 $\mu$M ouabain decreased the $V_B$ from $-75.5$ to $-49.5$ mV ($P < 0.01$). The responses of $\Delta V_{B_{K^+}}$ in the control and ouabain period were 7.3 and 7.8 mV, respectively ($P > 0.05$). The responses of $\Delta V_{B_{NH_4^+}}$ in comparable

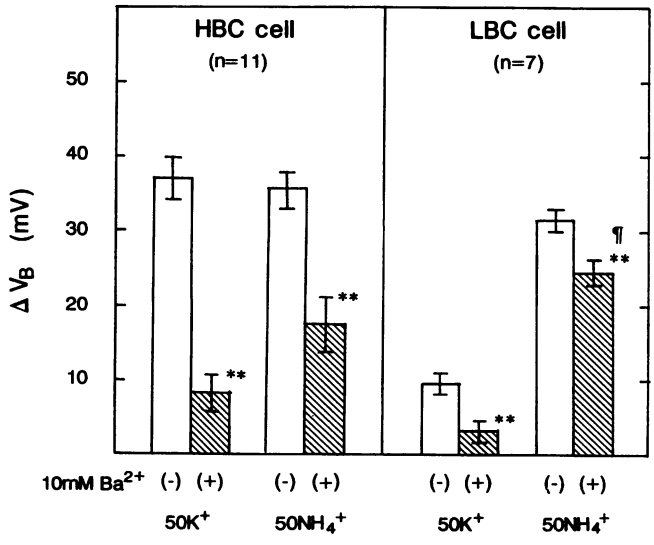


*Figure 6.* Summary of the data of experiments in which effects of $Ba^{2+}$ on $V_B$ responses to $50K^+$ or $50NH_4^+$ were observed. $^{**}P < 0.01$ compared to the values without $Ba^{2+}$. $\P P < 0.01$ compared to percent decrease in $\Delta V_{B_{NH_4^+}}$ in HBC cell.

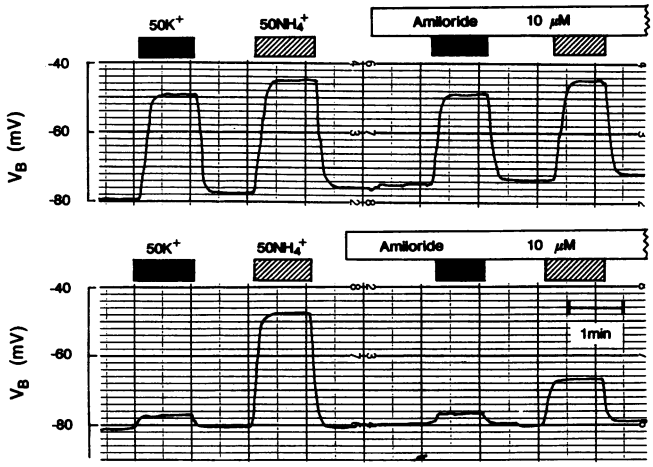


*Figure 7.* Representative tracings showing $V_B$ responses to $50K^+$ or $50NH_4^+$ in the presence or absence of amiloride in the bath. The upper panel represents the HBC cell, and the lower the LBC cell.

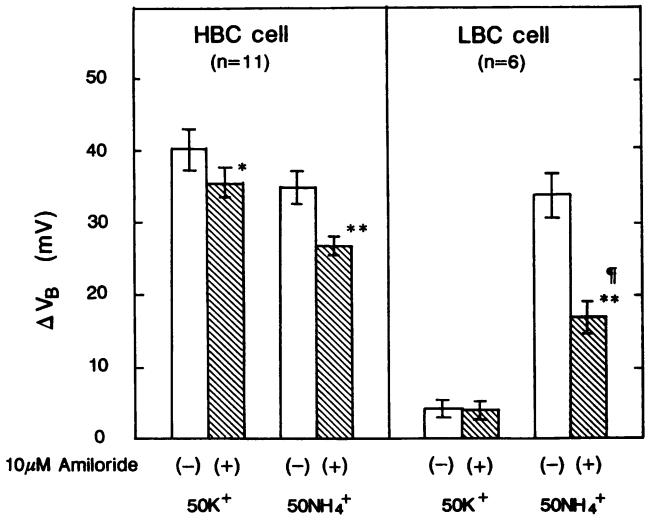


*Figure 8.* Summary of the data of experiments in which effects of amiloride on $V_B$ responses to $50K^+$ or $50NH_4^+$ were observed. $^{**}P < 0.05$, $P < 0.01$ compared to the values without amiloride. $\P P < 0.01$ compared to percent decrease in $\Delta V_{B_{NH_4^+}}$ in HBC cell.

periods were 41.8 and 40.3 mV, respectively ($P > 0.05$). These observations indicate that both $\Delta V_{B_{K^+}}$ and $\Delta V_{B_{NH_4^+}}$ were unaffected by ouabain in both cell types.

## Discussion

Ion transport mechanisms across the membranes of the thick ascending limb of Henle's loop have been well defined by extensive studies of Greger and his associates (17). According to the proposed model, the apical membrane is characterized by the existence of $Na^+$-$K^+$-$2Cl^-$ cotransporter and $K^+$ conductance, while the basolateral membrane is characterized by $Na^+$, $K^+$-pump, $K^+$ conductance, $Cl^-$ conductance, and $K^+$-$Cl^-$ cotransport. Yoshitomi et al. (9) reported that in the hamster MAL there are two cell types: one having a high basolateral membrane conductance (HBC cell) and the other having a low basolateral membrane conductance (LBC cell). These two cell types can be identified by the magnitude of the depolarization of the basolateral membrane upon abrupt increase in $K^+$ concentration in the bath. In other word, the HBC cell has a high basolateral $K^+$ conductance whereas the LBC cell has a low basolateral $K^+$ conductance.

In the present study, we identified these cell types by the magnitude of the $V_B$ deflection in response to a rapid increase in $K^+$ concentration in the bathing fluid from 5 to 50 mM. Based on the data from 101 intracellular impalements, we confirmed that there are two different types of cells with regard to the voltage response to an abrubt change in $K^+$ concentration of the bathing fluid from 5 to 50 mM. We demonstrated that the replacement of 50 mM $Na^+$ by $NH_4^+$ in the bathing fluid caused a rapid and reversible depolarization of the basolateral membrane of both cell types. However, in the HBC cell, the magnitude of the $V_B$ deflection upon 50 mM $NH_4^+$ was only slightly greater than that induced by 50 mM $K^+$. In contrast, in the LBC cell, the $V_B$ response to 50 mM $NH_4^+$ was much greater than that to 50 mM $K^+$. It is possible that the $V_B$ deflection elicited by the ion concentration change in the bathing fluid is influenced by the circular current through the paracellular shunt pathway. To estimate the contribution of this component, we conducted similar studies in the tubules of which lumen was completely collapsed. Although the voltage deflections observed under this condition were tended to be slightly higher, they were not statistically significant. Therefore, the contribution of the circular current through the paracellular shunt pathway may be very small if any.

*Table III. Effect of NPPB on Voltage Responses of the Basolateral Membrane to $50K^+$ or $50NH_4^+$ Challenge in the Bath in the Hamster MAL*

| | NPPB | HBC cell | LBC cell |
|---|---|---|---|
| *n* | | 10 | 6 |
| $V_B$ | C 0 | −75.1±4.8 | −68.5±3.3 |
| | E 10 μM | −81.7±4.7 | −77.7±2.5 |
| | E-C | −6.6±1.2‡ | −9.2±3.2* |
| $\Delta V_B(50K^+)$ | C 0 | 46.4±1.6 | 11.0±2.7 |
| | E 10 μM | 44.1±2.5 | 11.5±3.3 |
| | E-C | −2.3±2.1 | 0.5±2.3 |
| $\Delta V_B(50NH_4^+)$ | C 0 | 41.8±2.3 | 34.0±2.4 |
| | E 10 μM | 39.7±4.5 | 36.3±6.1 |
| | E-C | −2.1±2.6 | 2.3±3.7 |

Abbreviations and symbols are the same as in Table II.

*Table IV. Effect of Decrease or Elimination of $Na^+$ from the Bath on the Basolateral Membrane Voltage*

| | NaCl | HBC cell | LBC cell |
|---|---|---|---|
| | *mM* | | |
| *n* | | 5 | 4 |
| $V_B$ | C 211.6 | −67.8±3.8 | −73.8±4.4 |
| | E 20 | −70.0±3.9 | −74.0±4.3 |
| | E-C | −2.2±0.9 | −0.2±0.9 |
| *n* | | 5 | 4 |
| $V_B$ | C 211.8 | −74.2±4.6 | −70.3±4.0 |
| | E 0 | −73.6±4.3 | −69.3±4.3 |
| | E-C | −0.6±0.4 | 1.0±0.7 |

Abbreviations and symbols are the same as in Table II.

*Table V. Effect of Ouabain on Basal $V_B$ and on Voltage Responses of the Basolateral Membrane to $50K^+$ or $50NH_4^+$ Challenge in the Bath in the Hamster MAL*

| | Ouabain | HBC cell | LBC cell |
|---|---|---|---|
| | *μM* | | |
| *n* | | 7 | 4 |
| $V_B$ | C 0 | −73.0±1.9 | −75.5±2.9 |
| | E 10 | −48.2±3.3 | −49.3±4.2 |
| | E-C | −24.8±2.3‡ | −26.3±9.6* |
| $\Delta V_B(50K^+)$ | C 0 | 40.2±2.0 | 7.3±1.1 |
| | E 10 | 36.0±1.5 | 7.8±1.1 |
| | E-C | −4.2±2.2 | 0.5±1.3 |
| $\Delta V_B(50NH_4^+)$ | C 0 | 40.2±1.9 | 41.8±1.7 |
| | E 10 | 38.2±1.7 | 40.3±1.4 |
| | E-C | −2.0±1.8 | −1.5±1.7 |

Abbreviations and symbols are the same as in Table II. * $P < 0.01$; ‡ $P < 0.001$ as compared to zero.

There are several possible ways by which an increase in $NH_4^+$ concentration causes the basolateral membrane to depolarize. First, it is possible that this maneuver may inhibit elecetrogenic $N^+$, $K^+$-pump, leading to depolarization of the basolateral membrane. This possibility was excluded by the observation that the voltage deflection by $NH_4^+$ challenge was not affected by pretreatment of the tubule with ouabain.

Second, if the addition of $NH_4Cl$ to the bath causes cell pH to alkalinize by diffusion of $NH_3$, then intracellular $HCO_3^-$ increases and depolarizes the basolateral membrane through $Na^+$-$3HCO_3^-$ cotransporter. In the present study, we confirmed that there is a $HCO_3^-$ conductance also in the basolateral membrane of the hamster MAL (15, 16). However, the $V_B$ deflection by $NH_4Cl$ in the bath is not accounted for by this conductance. Because the most experiments were conducted in the absence of $HCO_3^-$ in ambient fluid, the contribution of $HCO_3^-$ conductance can be ruled out.

Third, it is also possible that cytosolic alkalinization might increase $Cl^-$ conductance in the basolateral membrane, causing depolarization of the basolateral membrane. This possibility was excluded by the observation that the $V_B$ deflection caused by $NH_4^+$ was unchanged in the presence of NPPB, a $Cl^-$ channel blocker (18). Under this experimental condition, NPPB may have had a definite inhibitory effect on the basolateral membrane $Cl^-$ conductance because it hyperpolarized the basolateral membrane.

Fourth, it is highly possible that $NH_4^+$ may depolarize the basolateral membrane by passing through a $K^+$ channel. The observation that in HBC cells the $V_B$ response to an increased $K^+$ concentration was only partially blocked by $Ba^{2+}$ suggests that a $Ba^{2+}$-insensitive $K^+$ conductance exists in the basolateral membrane of the HBC cells. Although the inhibitory effect of $Ba^{2+}$ on the $V_B$ deflection to $NH_4^+$ was less than that to $K^+$ in the HBC, it is impossible to conclude that there is a conductance specific to $NH_4^+$ in this cell type. By contrast, in the LBC cell the $V_B$ response to $NH_4^+$ was considerably high despite the fact that the response to $50K^+$ was very low. Moreover, 10 mM $Ba^{2+}$ added to the bath suppressed the $V_B$ response to $50K^+$ to a level that was not different from zero. Under this condition, the response to $50NH_4^+$ was decreased only by 23%. These observations are compatible with the view that the voltage deflection caused by an $NH_4^+$ gradient is not entirely accounted for by the electrodiffusion of $NH_4^+$ through a $K^+$ channel, but rather through a pathway specific for $NH_4^+$.

Bichara et al. (12) found an $NH_4^+$ conductance in the rat MAL fragments by measuring intracellular pH and voltage by fluorometry. However, it has not been determined whether the conductance was localized in the luminal or basolateral membrane. In the present study, we identified that the $NH_4^+$ conductance is located mainly in the basolateral membrane of the LBC cell. Bichara et al. (12) also reported that 1 $\mu$M amiloride partially inhibited the $NH_4^+$ conductance. However, because Discala et al. (19) recently reported that a millimolar concentration of amiloride blocks $K^+$ conductance of the apical membrane of *Necturus* proximal tubular cells, the interpretation of the data on amiloride is somewhat difficult. Nevertheless, the observations that in the LBC cell 10 $\mu$M amiloride inhibited the $V_B$ response to $50NH_4^+$ without affecting the response to $50K^+$ favor the view that the inhibitory effect of amiloride was more specific for the putative $NH_4^+$ conductance in the LBC cell.

It is of interest to note that similar $NH_4^+$ conductances were also found in rabbit proximal straight tubules (10) and *Xenopus* oocytes (13, 20). Burckhardt and Frömter (13) suggested that in *Xenopus* oocytes $NH_4^+$ may pass through a nonselective cation channel because the conductance was inhibited by various agents which are known to inhibit the nonselective cation channels. In the present study, however, we could not demonstrate any appreciable $Na^+$ conductance in the basolateral membrane of both cell types of the hamster MAL. Therefore, the $NH_4^+$ conductance in the hamster MAL may be distinct from the nonselective cation conductance.

Although it is unequivocal that there is an $NH_4^+$ conductance in the basolateral membrane of the LBC cell, the physiological significance of this conductance is unknown at present time. It is unlikely that this is a route of $NH_4^+$ exit at the basolateral membrane because an electrochemical gradient may be unfavorable for the $NH_4^+$ exit across the basolateral membrane. Therefore, it is possible that the putative $NH_4^+$ conductance of the basolateral membrane might act to reduce the net lumen to bath $NH_4^+$ flux by allowing the back flux of $NH_4^+$ from the bath to cytoplasm. In this regard, it should be noted that the conductance is predominant in the LBC cell. Because of the high $NH_4^+$ conductance in the basolateral membrane, $NH_4^+$ concentration in the LBC cell might be higher than that in the HBC cell. This would in turn reduce the driving force for $NH_4^+$ entry across the apical membrane through $Na^+$-$NH_4^+$-$2Cl^-$ cotransport. Functional significance of the cell heterogeneity in the MAL is unknown at present. Yoshitomi et al. (9) proposed that the LBC cell may participate in $K^+$ secretion whereas the HBC cell reabsorbs $K^+$. Along the same line, we speculate that the HBC cell may participate in $NH_4^+$ reabsorption whereas the LBC cell may act to suppress $NH_4^+$ reabsorption. It is difficult to assess whether the latter participates in $NH_4^+$ secretion without knowing the mechanisms of $NH_4^+$ exit across the apical membrane. Further studies are obviously necessary to elucidate the functional significance of the $NH_4^+$ conductance in the basolateral membrane of the LBC cell of the hamster MAL.

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