Evidence for ammonium conductance in a mouse thick ascending limb cell line.

Soojung Lee, Emory University
Jonathan Park, Emory University
Jun Min Li, Emory University
Kathy Li, Emory University
Inyeong Choi, Emory University

Journal Title: Physiological Reports
Volume: Volume 5, Number 16
Publisher: Wiley Open Access | 2017-08-21
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.14814/phy2.13379
Permanent URL: https://pid.emory.edu/ark:/25593/s4v9w

Final published version: http://dx.doi.org/10.14814/phy2.13379

Copyright information:
© 2017 The Authors. Physiological Reports published by Wiley Periodicals, Inc. on behalf of The Physiological Society and the American Physiological Society
This is an Open Access work distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/).

Accessed June 20, 2022 4:00 PM EDT
Evidence for ammonium conductance in a mouse thick ascending limb cell line

Soojung Lee, Jonathan Park, Jun Ming Li, Kathy Li & Inyeong Choi
Department of Physiology, Emory University School of Medicine, Atlanta, Georgia

Keywords
Ammonium conductance, thick ascending limb, Xenopus oocytes.

Correspondence
Inyeong Choi, Department of Physiology, Emory University School of Medicine, Atlanta, GA 30322.
Tel: 404-712-2092
Fax: 404-727-2648
E-mail: ichoi@emory.edu

Funding Information
Whole cell recordings were performed with the patch clamp equipment purchased using the NIH supplement for instrument and thus the study was partly supported by the NIH R01 GM078502-04-S to Choi.

Received: 10 March 2017; Revised: 23 June 2017; Accepted: 14 July 2017
doi: 10.14814/phy2.13379
Physiol Rep, 5 (16), 2017, e13379, https://doi.org/10.14814/phy2.13379

Abstract
In this study, we examined an ammonium conductance in the mouse thick ascending limb cell line ST-1. Whole cell patch clamp was performed to measure currents evoked by NH4Cl in the presence of BaCl2, tetrathylammonium, and BAPTA. Application of 20 mmol/L NH4Cl induced an inward current (∆-272 ± 79 pA, n = 9). In current-voltage (I–V) relationships, NH4Cl application caused the I–V curve to shift down in an inward direction. The difference in current before and after NH4Cl application, which corresponds to the current evoked by NH4Cl, was progressively larger at more negative potentials. The reversal potential for NH4Cl was +15 mV, higher than the equilibrium potential for chloride, indicating that the current should be due to NH4+. We then injected ST-1 poly(A) RNA into Xenopus oocytes and performed two-electrode voltage clamp. NH4Cl application in the presence of BaCl2 caused the I–V curve to be steeper. The NH4+ current was retained at pH 6.4, where endogenous oocyte current was abolished. The NH4+ current was unaffected by 10 μmol/L amiloride but abolished after incubation in Na+-free media. These results demonstrate that the renal cell line ST-1 produces an NH4+ conductance.

Introduction
NH4+ is a key buffer component that regulates blood pH. In essence, the kidneys excrete NH4+ to urine as they produce HCO3−, and the mechanism by which NH4+ excretion results in net acid excretion involves a series of sophisticated NH4+ transport processes in different parts of the nephron (Weiner and Verlander 2013; Hamm et al. 2015). One of the nephron segments that play key roles in NH4+ excretion is the thick ascending limb (TAL) (Mount 2014). NH4+ transport in the TAL involves the Na/K/2Cl cotransporter NKCC2 (Good et al. 1984; Kinne et al. 1986), K/NH4 exchange and NH4+ conductance (Amlal et al. 1994; Attmane-Elakeb et al. 2001) in the luminal membrane of the tubule, and the Na/H exchanger NHE4 (Bourgeois et al. 2010) in the basolateral membrane. The basolateral NH4+ transport is also mediated by a dissociation of intracellular NH4+ into NH3 and H+ and subsequent NH3 exit to the interstitium.

In the luminal membrane of the TAL, NKCC2 is the major fraction of the active NH4+ flux. Nonetheless, in vitro studies reveal that K/NH4 exchange and NH4+ conductance can contribute to the NH4+ transport by 35–50% (Amlal et al. 1994; Attmane-Elakeb et al. 2001). K/NH4 exchange is barium- and verapamil-sensitive, whereas NH4+ conductance is barium-insensitive and amiloride-sensitive (Amlal et al. 1994). The two pathways exhibit biophysical and pharmacological characteristics that distinguish them from other NH4+-transporting proteins. Despite such physiological and functional
significance, our understanding of these pathways is limited because their molecular entities are presently unknown.

In this study, we examined an NH$_4^+$ conductance in the mouse TAL cell line ST-1. This cell line is nonpolarized and exhibits many features characteristic of TAL cells (Kone et al. 1995; Kone and Higham 1999; Lee et al. 2010). We performed whole cell patch clamp of the cells to identify the NH$_4^+$ conductance and determine basic electrophysiological properties such as the amount of current, direction, current-voltage relationship, and reversal potential. We then isolated ST-1 poly(A) RNA and injected it into Xenopus oocytes and performed two-electrode voltage clamp in an effort to identify a protein conducting NH$_4^+$. While our search for the protein is in progress, here we report that the NH$_4^+$ conductance in ST-1 cells is not identical to the previously reported Cl$^-$-dependent NH$_4^+$ conductance in the TAL of the nephron.

**Methods**

**Ethical approval**

All experiments in this study were conducted under the National Institutes of Health guidelines for research, and experimental protocols were approved by the Institutional Animal Care and Use Committee at Emory University.

**Cell culture**

ST-1 is a cell line derived from mouse medullary TAL tubules, developed by Bruce Kone (Kone et al. 1995; Kone and Higham 1999). Cell authentication is based on the report (Haas and Hebert 1992) on the expression of bumetanide-sensitive proteins and our previous report (Lee et al. 2010) demonstrating the expression of the electroneutral Na/HCO$_3^-$ transporter NBCn1 and Cl/HCO$_3^-$ exchanger AE2 in this cell line by immunoblot. Cells were cultured in Dulbecco’s modified eagle’s medium supplemented with 10% fetal bovine serum, 50 U/mL penicillin and 50 µg/mL streptomycin in a 5% CO$_2$ air equilibrated 37°C incubator. For patch clamp recording, cells were seeded on poly-D-lysine-coated coverslips at a density of $8 \times 10^4$ cells per well in a 12-well plate. Recordings were done 2 days later.

**Whole cell patch clamp of ST-1 cells**

Whole cell recording was performed using the protocol by Hayashi et al. (1992) with slight modification. A coverslip on which cells were grown was mounted on a recording chamber affixed on the stage of a Nikon Eclipse T1 inverted microscope, equipped with a MP-225 motorized micromanipulator. Cells were perfused with solution containing (in mmol/L) 116 NaCl, 6 KCl, 2.4 CaCl$_2$, 6 glucose, 10 HEPES, 10 tetraethylammonium (TEA), and 1 BaCl$_2$, pH 7.4. The pipette had 116 KCl, 1.2 MgCl$_2$, 6 glucose, 10 HEPES, 10 TEA, 10 BAPTA, and 0.01 BaCl$_2$ (pH 7.2). Recording was made using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale). Currents were recorded with 20 mmol/L NH$_4$Cl, at a holding voltage of ~70 mV. For amiloride experiments, currents induced by NH$_4$Cl were recorded in the absence and then presence of 1 µmol/L amiloride (Sigma-Aldrich; Cat#: A7410). $I-V$ relationship was determined by a staircase voltage command between −80 to +15 mV (170 msec duration). Currents were recorded using pClamp 8.0 (Molecular Devices) and signals were low-pass filtered (3 db at 2 kHz, 8-pole Bessel filter). Experiments were performed at room temperature.

**Two-electrode voltage clamp of oocytes**

ST-1 cells grown in 100 mm plates were collected and lysed for poly(A) RNA isolation using RNeasy Mini kit (Qiagen, Germantown). *Xenopus laevis* oocytes at stages V and VI were purchased from Ecocyte Bioscience (Austin). Poly(A) RNA was injected into oocytes (2.7 ng per oocyte in 46 nl) and controls were water only. Oocytes were maintained for 3 days at 18°C before use. A RC-24N recording chamber (Warner Instrument, Hamden) was filled with perfusion solution containing (in mmol/L) 20 LiCl, 80 NaCl, 3.8 BaCl$_2$, and 5 HEPES, pH 7.4. For acidic solution, the pH was adjusted to 6.4. An oocyte was placed in the chamber and impaled with two glass electrodes filled with 3 mol/L KCl (a tip resistance of 0.5–2 mol/LΩ). The oocyte was clamped at −60 mV using a OC-725C voltage-clamp amplifier (Warner Instrument). NH$_4$Cl solutions were made by replacing LiCl at equimolar concentrations. Na$^+$-free solutions were made by replacing Na$^+$ with N-methyl-D-glucamine (NMDG). For drug sensitivity experiments, 10 µmol/L amiloride and 200 µmol/L bumetanide (Sigma-Aldrich; Cat #: B3023) were used. The voltage command was from −140 to +40 mV with 20 mV increments (100 msec duration). The voltage command after NH$_4$Cl application was made when the current reached steady state (~4 min) (Lee and Choi 2011). Voltage signals were sampled by a Digidata 1322A (Molecular Devices) and data were acquired using pClamp 8.0.

**Statistical analysis**

Data were reported as mean ± standard error. The level of significance was determined using (1) paired, two-tailed Student $t$-test for comparison of slopes before and after NH$_4^+$ application to oocytes or ST- cells, and comparison of the currents before and after NH$_4$Cl
application in patch clamp; (2) unpaired, two-tailed Student t-test for comparison of \( \Delta \)NH\(_4\) between RNA-injected versus water-injected oocytes; and (3) two-way ANOVA with Bonferroni post hoc test for comparison of slopes in the presence of bumetanide and in Na\(^+\)-free solutions between different groups of oocytes. The \( P \) value of less than 0.05 was considered significant. Analysis was made using Microsoft Office Excel add-in program Analysis ToolPak (Redmond, WA).

**Results**

**NH\(_4^+\) conductance in ST-1 cells**

We performed patch clamp recording of ST-1 cells in a whole cell configuration to assess NH\(_4^+\) conductance. Recordings were performed in the presence of 1 mmol/L BaCl\(_2\) and 10 mmol/L TEA to block K channels, and 5 mmol/L BAPTA to block intracellular Ca\(^{2+}\) increase. Figure 1A shows an example of the inward current evoked by 20 mmol/L NH\(_4\)Cl at the holding potential of −70 mV. As in many cells, the current was decayed slowly but we also frequently observed steady-state currents after reaching a peak. Figure 1B shows the currents measured before and after NH\(_4\)Cl application. The mean difference between the two values, corresponding the current evoked by NH\(_4\)Cl, was −272 ± 79 pA \((P < 0.05; n = 9)\). In \( I-V \) relationships (Fig. 1C), NH\(_4\)Cl application caused the \( I-V \) curve to shift down in an inward direction. The difference in the two curves, corresponding to the currents evoked by NH\(_4\)Cl at different voltages, was progressively larger at more negative potentials.
The reversal potential for NH$_4$Cl was $+15 \text{ mV}$, higher than the equilibrium potential for chloride ($-4 \text{ mV}$) estimated by chloride concentrations in the patch pipette and bath solutions. Thus, the inward current evoked by NH$_4$Cl is not due to Cl$^-$, but to NH$_4^+$. The NH$_4^+$ conductance ($G_{NH4}$) determined by the difference in the slopes of the two $I-V$ curves was 4.4 nS (Fig. 1D). In other experiments, we determined the effect of amiloride on the currents (Fig. 1E). Measured at the holding potential of $-70 \text{ mV}$, the currents were unaffected by 1 $\mu$mol/L amiloride ($P > 0.05$; $n = 6$).

To express ST-1 proteins in *Xenopus* oocytes, we isolated poly(A) RNA from the cells and injected it into oocytes. Figure 2A shows $I-V$ relationships of water-injected control oocytes, obtained before and after application of 20 mmol/L NH$_4$Cl in the presence of 3.8 mmol/L BaCl$_2$. NH$_4$Cl caused the $I-V$ curve to shift down in an inward direction. This shift is due to endogenous oocyte conductance (Lee and Choi 2011). A slight increase in an outward current was observed, probably due to LiCl that depolarizes oocyte membranes. In oocytes injected with...
poly(A) RNA (Fig. 2B), the basal current before NH₄Cl application was higher, due to Na⁺ as described below. NH₄Cl caused the I–V curve to shift down with a steeper slope. At /C₀ 60 mV, the inward current in these oocytes was /C₀ 780 ± 164 nA, significantly higher than /C₀ 240 ± 12 nA in controls. The reversal potential (the voltage where the two I–V curves intersect) was similar between control oocytes and RNA-injected oocytes, indicating that the currents are likely produced by nonselective cation channels. Figure 2C shows the comparison of the slopes determined near the reversal potential. RNA-injected oocytes had a more increased slope in response to NH₄Cl (P < 0.05 for both; n = 7 for RNA-injected oocytes and 5 for water-injected controls). Thus, the G₉NH₄ in RNA-injected oocytes was significantly larger than that of controls (P < 0.05; Fig. 2D). In other experiments, we examined the effect of amiloride on the slope induced by NH₄Cl (Fig. 2E). We found no significant change by 10 μmol/L amiloride (P > 0.05; n = 5 for each).

Inhibition of G₉NH₄ by incubation in Na⁺-free solutions

To test whether the G₉NH₄ in RNA-injected oocytes is affected by Na⁺, we incubated oocytes in Na⁺-free solutions (NMDG replaced Na⁺) and determined I–V relationships. The incubation time was at least 3 h to ensure that intracellular Na⁺ is substantially low to minimize Na⁺ efflux. Figure 4A shows the I–V relationships under Na⁺-free conditions. NH₄Cl application caused the I–V curve of control oocytes to inwardly shift down, indicating negligible effect of Na⁺ removal on endogenous conductance. In contrast, Na⁺ removal induced two changes in RNA-injected oocytes. First, the I–V curve before NH₄Cl application was similar to the control curve. Second, the shift in the curve after NH₄Cl application was smaller than the control. These changes were evident when the slopes of the curves were compared (Fig. 4B). Compared to controls, RNA-injected oocytes had a similar slope of the basal current (P > 0.05; n = 7 for RNA-injected oocytes and 6 for controls) but a small slope of the NH₄⁺ current (P < 0.05). This resulted in a smaller G₉NH₄ in RNA-injected oocytes (Fig. 4C). To test whether the ‘below-control’ decrease in G₉NH₄ is associated with NKCC2, we treated oocytes with 200 μmol/L bumetanide under Na⁺-free conditions. Figure 4D shows an example of I–V relationships after treating with bumetanide. The I–V curves after NH₄Cl application were nearly superimposed between controls and RNA-injected oocytes, indicating that bumetanide unleashed the excessive inhibition of the G₉NH₄ by Na⁺ removal. The two groups of oocytes had similar slopes and G₉NH₄ (P < 0.05; n = 6 for RNA-injected oocytes and 7 for controls), as

Retention of ST-1 G₉NH₄ at acidic pH

We performed I–V recordings in solutions with pH 6.4 to determine whether endogenous oocyte conductance was responsible for increased G₉NH₄ in RNA-injected oocytes. Endogenous oocyte NH₄⁺ transport is known to be inhibited at low pH (Nakhoul et al. 2010). We found that while the G₉NH₄ in control oocytes was abolished at pH 6.4, the one in RNA-injected oocytes was still induced under the same condition (P < 0.05; Fig. 3). Thus, the G₉NH₄ in RNA-injected oocytes is mainly induced by heterologously expressed ST-1 proteins.

© 2017 The Authors. Physiological Reports published by Wiley Periodicals, Inc. on behalf of The Physiological Society and the American Physiological Society

Figure 3. NH₄⁺ conductance at pH 6.4. (A) I–V relationships recorded at pH 6.4. Recordings were made using the protocol described in Figure 2. Data are from 5 RNA-injected oocytes and 5 controls. (B) Mean slope of the I–V curve. Slopes were determined using the I–V relationships in A.
demonstrated in Figure 4E and F. We also used 500 μmol/L of bumetanide and found the same effects (data not shown).

Discussion

The aim of this study was to obtain the basic electrophysiological properties of the NH$_4^+$ conductance in the TAL cell line ST-1 for future study of its molecular identification. Using a homogeneous cell line and its protein expression in a relatively simple heterologous oocyte system, we identified an NH$_4^+$ conductance and obtained information on the direction of the current flow, amounts of currents produced, I–V relationships, and ion dependence. An interesting finding is that this conductance is not inhibited by amiloride and appears to be dependent upon Na$^+$. This finding is significant as it provides evidence that the NH$_4^+$ conductance in ST-1 is different from the previously reported Cl$^-$-dependent NH$_4^+$ conductance in the TAL of a nephron.

Amlal et al. (1994) have reported the NH$_4^+$ conductance in the isolated rat medullary TAL by monitoring membrane depolarization of the tubules using DiSC$_3$(5). While this approach determines whether there is a current flowing across the membrane, it does not measure actual membrane conductance. Current is proportional to conductance at a constant voltage, and a voltage or current clamp should be done to correctly measure a membrane conductance. In our study, we directly measured the current evoked by NH$_4$Cl. The current was inwardly directed.
with a positive reversal potential and a positive $G_{\text{NH}_4}$ was observed. These data are consistent with an inward movement of positively charged $\text{NH}_4^+$ ions. This inward current is unlikely mediated by $\text{Cl}^-$. Given the $\text{Cl}^-$ concentrations in the patch pipette and in the bath, the equilibrium potential for $\text{Cl}^-$ is estimated to be slightly negative. The voltage-clamp experiments of oocytes shows that the $\text{NH}_4^+$ conductance is produced in the presence of barium. Patch clamp of ST-1 cells also shows $\text{NH}_4^+$ conductance in the presence of barium and TEA, consistent with the report that $\text{NH}_4^+$ is poorly transported by K channels in the TAL (Attmane-Elakeb et al. 2001).

We found that the $G_{\text{NH}_4}$ was inhibited after incubation in Na$^+$-free solutions (Fig. 4). This Na$^+$-dependent inhibition is unexpected because an $\text{NH}_4^+$ current is considered to occur via K$^+$ channels (or transporters) by replacing a K binding site. One explanation is that Na$^+$-free incubation has lowered intracellular pH by reversing the Na/H exchangers and then inhibited the $G_{\text{NH}_4}$. However, this is unlikely to happen because the $G_{\text{NH}_4}$ can be produced at acidic pH as shown in Figure 3. Another explanation is that Na$^+$-free solutions has inhibited Na$^+$ channels or Na$^+$ channel-like proteins, which may induce $\text{NH}_4^+$ currents. NH$^+_2$ affects activities of epithelial sodium channel ENaC (Nakhoul et al. 2001) and gates acid-sensing ion channel ASICs (Pidoplichko and Dani 2006). Nonetheless, we do not think that ENaC and ASICs are responsible for the $G_{\text{NH}_4}$ in ST-1 because these two proteins are very sensitive to amiloride (Benos 1982; Wemmie et al. 2006). We found no amiloride sensitivity of the $G_{\text{NH}_4}$. The $\text{NH}_4^+$ conductance in the TAL is sensitive to amiloride given that 1 mmol/L amiloride completely abolishes the NH$^+_4$Cl-induced membrane depolarization in the isolated TAL tubules (Amlal et al. 1994). The TAL does not express ENaC although we note that ENaC antibodies detect signals in the luminal side of rat TAL (Brown et al. 1989). Taken together, we think that the molecule responsible for $\text{NH}_4^+$ conductance in ST-1 is a novel protein that is not inhibited by amiloride and has sensitivity to Na$^+$, probably to intracellular Na$^+$. ST-1 RNA-injected oocytes do not retain $G_{\text{NH}_4}$ after incubation in Na$^+$-free solutions (S. Lee, unpublished observation), implying that the intracellular Na$^+$ levels are critical for $G_{\text{NH}_4}$.

What would be a potential role of the $G_{\text{NH}_4}$ in renal ammonium excretion? We think that the conductance would contribute to renal adaptive process in acid-base disorders. For example, in rats and humans, K$^+$ depletion is associated with increased urinary $\text{NH}_4^+$ production and excretion, ultimately developing metabolic alkalosis (Jones et al. 1982; Abu Hossain et al. 2011). While this development is probably due to increased ammoniagenesis in the proximal tubules, K$^+$ depletion downregulates NKCC2 in the TAL (Amlal et al. 1998). Thus, it is possible that other mechanisms such as $\text{NH}_4^+$ conductance are upregulated during K$^+$ depletion. In addition, the $G_{\text{NH}_4}$ may serve as a fine-tuning regulator of $\text{NH}_4^+$ transport in the TAL, where luminal $\text{NH}_4^+$ transport is mainly mediated by electroneutral NKCC2. The regulation of $G_{\text{NH}_4}$ by Na$^+$ might be a novel mechanism that links NKCC2 to $\text{NH}_4^+$ absorption and subsequent excretion.

In conclusion, our findings are interesting and provide a foundation for future studies of electrophysiological and pharmacological properties of the $\text{NH}_4^+$ conductance. Those data will subsequently lead to obtaining molecular information on an ammonium channel.

Acknowledgment

We appreciate Douglas Eaton for helpful discussion on patch clamp data.

Conflict of Interest

None declared.

References

Abu Hossain, S., F. A. Chaudhry, K. Zahedi, F. Siddiqui, and H. Amlal. 2011. Cellular and molecular basis of increased ammoniagenesis in potassium deprivation. Am. J. Physiol. Renal Physiol. 301:F969–F978.

Amlal, H., M. Paillard, and M. Bichara. 1994. NH$^+_4$ transport pathways in cells of medullary thick ascending limb of rat kidney. NH$^+_4$ conductance and K$^+$/NH$^+_4$(H$^+$) antiport. J. Biol. Chem. 269:21962–21971.

Amlal, H., Z. Wang, and M. Soleimani. 1998. Potassium depletion downregulates chloride-absorbing transporters in rat kidney. J. Clin. Invest. 101:1045–1054.

Attmane-Elakeb, A., H. Amlal, and M. Bichara. 2001. Ammonium carriers in medullary thick ascending limb. Am. J. Physiol. Renal Physiol. 280:F1–F9.

Benos, D. J. 1982. Amiloride: a molecular probe of sodium transport in tissues and cells. Am. J. Physiol. 242:C131–C145.

Bourgeois, S., L. V. Meer, B. Wootla, M. Bloch-Faure, R. Chambrey, G. E. Shull, et al. 2010. NHE4 is critical for the renal handling of ammonia in rodents. J. Clin. Invest. 120:1895–1904.

Brown, D., E. J. Sorscher, D. A. Ausiello, and D. J. Benos. 1989. Immunocytochemical localization of Na$^+$ channels in rat kidney medulla. Am. J. Physiol. 256:F366–F369.

Good, D. W., M. A. Knepper, and M. B. Burg. 1984. Ammonia and bicarbonate transport by thick ascending limb of rat kidney. Am. J. Physiol. 247:F35–F44.

Haas, M., and S. C. Hebert. 1992. [3H]bumetanide binding to a mouse medullary thick limb (MTAL) cell line (Abstract). J. Am. Soc. Nephrol. 3:808.
Hamm, L. L., N. Nakhoul, and K. S. Hering-Smith. 2015. Acid-base homeostasis. Clin. J. Am. Soc. Nephrol. 10:2232–2242.

Hayashi, T., T. Shigetomi, M. Ueda, T. Kaneda, T. Matsumoto, H. Tokuno, et al. 1992. Effects of ammonium chloride on membrane currents of acinar cells dispersed from the rat parotid gland. Pflugers Arch. 420:297–301.

Jones, J. W., A. Sebastian, H. N. Hulter, M. Schambelan, J. M. Sutton, and E. G. Biglieri. 1982. Systemic and renal acid-base effects of chronic dietary potassium depletion in humans. Kidney Int. 21:402–410.

Kinne, R., E. Kinne-Saffran, H. Schutz, and B. Scholermann. 1986. Ammonium transport in medullary thick ascending limb of rabbit kidney: involvement of the Na⁺, K⁺, Cl⁻ cotransporter. J. Membr. Biol. 94:279–284.

Kone, B. C., and S. Higham. 1999. Nitric oxide inhibits transcription of the Na⁺-K⁺-ATPase α1-subunit gene in an MTAL cell line. Am. J. Physiol. Renal Physiol. 276:F614–F621.

Kone, B. C., J. Schwobel, P. Turner, M. G. Mohaupt, and C. B. Cangro. 1995. Role of NF-kappa B in the regulation of inducible nitric oxide synthase in an MTAL cell line. Am. J. Physiol. 269:F718–F729.

Lee, S., and I. Choi. 2011. Sodium-bicarbonate cotransporter NBCn1/Slc4a7 inhibits NH₄Cl-mediated inward current in Xenopus oocytes. Exp. Physiol. 96:745–755.

Lee, S., H. J. Lee, H. S. Yang, I. M. Thornell, M. O. Bevensee, and I. Choi. 2010. Sodium-bicarbonate cotransporter NBCn1 in the kidney medullary thick ascending limb cell line is upregulated under acidic conditions and enhances ammonium transport. Exp. Physiol. 95:926–937.

Mount, D. B. 2014. Thick ascending limb of the loop of Henle. Clin. J. Am. Soc. Nephrol. 9:1974–1986.

Nakhoul, N. L., K. S. Hering-Smith, S. M. Abdulnour-Nakhoul, and L. L. Hamm. 2001. Ammonium interaction with the epithelial sodium channel. Am. J. Physiol. Renal Physiol. 281:F493–F502.

Nakhoul, N. L., S. M. Abdulnour-Nakhoul, E. Schmidt, R. Doetjes, E. Rabon, and L. L. Hamm. 2010. pH sensitivity of ammonium transport by Rhgb. Am. J. Physiol. Cell Physiol. 299:C1386–C1397.

Pidoplichko, V. I., and J. A. Dani. 2006. Acid-sensitive ionic channels in midbrain dopamine neurons are sensitive to ammonium, which may contribute to hyperammonemia damage. Proc. Natl Acad. Sci. USA 103:11376–11380.

Weiner, I. D., and J. W. Verlander. 2013. Renal ammonia metabolism and transport. Compr. Physiol. 3:201–220.

Wemmie, J. A., M. P. Price, and M. J. Welsh. 2006. Acid-sensing ion channels: advances, questions and therapeutic opportunities. Trends Neurosci. 29:578–586.