Aging Cell (2006) **5**, pp367–372 Doi: 10.1111/j.1474-9726.2006.00229.x

Relative contribution of V-H^+ATPase and NA^+/H^+ exchanger to bicarbonate reabsorption in proximal convoluted tubules of old rats

Mariana Fiori,[1,5] Martín Radrizzani,[3,4] Paula Díaz-Sylvester,[1] Angélica Müller,[2] Tomás Corti,[1] Alberto Monserrat[5] and Carlos Amorena[1]

[1]Escuela de Ciencia y Tecnología, Universidad Nacional de Gral. San Martín, San Lorenzo 3391, (1650) San Martín, Argentina
[2]Instituto de Investigaciones Cardiológicas (CONICET), Marcelo T. de Alvear 2270, (1122) Buenos Aires, Argentina
[3]Fundación Instituto Leloir, (IIBBA-CONICET, IIB-FCEN-UBA), Patricias Argentinas 435, (1405) Buenos Aires, Argentina
[4]Centro Nacional de Genética Medica, ANLIS-Dr. Carlos G. Malbrán, Las Heras 2670 4°, (1425) Buenos Aires, Argentina
[5]Centro de Patología Experimental. Departamento de Patología, Facultad de Medicina. Universidad de Buenos Aires. J. E. Uriburu 950 5°, (1114) Buenos Aires, Argentina

Summary

With aging, the kidney develops a progressive deterioration of several structures and functions. Proximal tubular acidification is impaired in old rats with a decrease in the activity of brush border Na^+/H^+ exchange and a fall of H-ion flux measured with micropuncture experiments. In the present work we evaluate the contribution of 5-N-ethyl-n-isopropyl amiloride- (EIPA) and bafilomycin-sensitive bicarbonate flux ($J_{HCO_3^-}$) in proximal convoluted tubules of young and aged rats. We performed micropuncture experiments inhibiting the Na^+/H^+ exchanger with EIPA (10^{-4} M) and the V-H^+ATPase with bafilomycin (10^{-6} M). We used antibodies against the NHE3 isoform of the Na^+/H^+ exchanger and the subunit E of the V-H^+ATPase for detecting by Western blot the abundance of these proteins in brush border membrane vesicles from proximal convoluted tubules of young and old rats. The abundance of NHE3 and the V-H^+ATPase was similar in 18-month-old and 3-month-old rats. The bicarbonate flux in old rats was 30% lower than in young rats. EIPA reduced $J_{HCO_3^-}$ by 60% and bafilomycin by 30% in young rats; in contrast, EIPA reduced $J_{HCO_3^-}$ by ~40% and bafilomycin by ~50% in old rats. The $J_{HCO_3^-}$ inhibited by bafilomycin was the same in young and old rats: 0.62 nmol · cm^{-2} · s^{-1} and 0.71 nmol · cm^{-2} · s^{-1}, respectively. However, the EIPA-sensitive fraction was larger in young than in old rats: 1.26 nmol · cm^{-2} · s^{-1} vs. 0.85 nmol · cm^{-2} · s^{-1}, respectively. These results suggest that the component more affected in bicarbonate reabsorption of proximal convoluted tubules from aged rats is the Na^+-H^+ exchanger, probably a NHE isoform different from NHE3.

Key words: aging; kidney; micropuncture experiments; Na^+/H^+ exchange; V-H^+-ATPase; Western blot.

Introduction

As part of the normal aging process, the kidney exhibits a progressive deterioration in several structures and functions (Davies & Shock, 1950; Baylis, 1994). Glomerular filtration rate, renal blood flow and concentrating ability decrease with age (Papper, 1973; Corman *et al*., 1985; Lindeman *et al*., 1985; Lindeman, 1995; Reilly *et al*., 1995). Furthermore, morphological changes appear, which include focal and segmental glomerular esclerosis and a reduction in the number of functioning glomeruli (Baylis, 1994). There is down-regulation of the renin-angiotensin system (RAS) with age, affecting renin mRNA and angiotensin-converting enzyme levels (Jung *et al*., 1995). In addition, renal hemodynamics in old rats seems to be more dependent on endothelium-derived relaxing factor (EDRF) than in young rats (Hill *et al*., 1997). Kinsella & Sacktor (1987) found a decrease of Na^+/H^+ exchanger activity in brush border membrane vesicles (BBMV) from the renal cortex of old animals. In a previous work, we found that proximal tubular acidification is impaired in old rats, with a decrease in the activity of Na^+/H^+ exchanger measured in BBMV and a fall of H-ion flux measured with micropuncture experiments (MacLaughlin *et al*., 2001). However, we did not identify what mechanisms were responsible for the reduction of H-ion flux observed in the proximal tubules of old rats. Under normal conditions, Na^+/H^+ exchange accounts for the major part of the proximal tubular acidification and a significant fraction (~30%) is mediated by a V-H^+ATPase (Bank *et al*., 1985; Preisig *et al*., 1987; Choi *et al*., 2000; Wang *et al*., 2001). The control of the exchanger is very complex and depends on many factors including, among others, RAS (Reilly *et al*., 1995), EDRF (Amorena & Castro, 1997; Manning *et al*., 1997; Wang, 1997), and parathyroid hormone (Bank & Aynedjain, 1976). The V-H^+ATPase is an electrogenic proton pump, sensitive to bafilomycin (Nakhoul & Hamm, 2002), which couples hydrolysis of cytosolic ATP to proton extrusion out of the cytosol to the lumen (Nelson & Harvey, 1999).

The aim of the present work is to evaluate the relative contribution of the Na^+/H^+ exchanger and the V-H^+ATPase to

Correspondence
Carlos E. Amorena, ECyT-UNSAM ED 23 GRAL PAZ 5445(1650) San Martin, BSAS, Argentina. Tel.: 54-11-4580-7289; fax: 54-11-4580-7296 106; e-mail: carlos.amorena@unsam.edu.ar

Accepted for publication *13 May 2006*

© 2006 The Authors
Journal compilation © Blackwell Publishing Ltd/Anatomical Society of Great Britain and Ireland 2006

Table 1 t/2 (0.693/k), sspH (steady state pH) and $J_{HCO_3^-}$ (bicarbonate flux) in 3-month- and 18-month-old rats either untreated (controls), or following treatment with 5-N-ethyl-n-isopropyl amiloride (EIPA, 10^{-4} M) or Bafilomycin(10^{-6} M). Values are means ± SEM

Experimental groups (no. of curves)	t/2 (s)	sspH	$J_{HCO_3^-}$ (nmol cm^{-2} s^{-1})
Control 3-month (39)	4.79 ± 0.31	6.89 ± 0.012	2.12 ± 0.16
Control 18-month (41)	7.90 ± 0.61	6.82 ± 0.010	1.46 ± 0.12*
EIPA 3-month (39)	11.27 ± 0.72	6.92 ± 0.010	0.86 ± 0.05*
EIPA 18-month (37)	21.84 ± 1.51	6.81 ± 0.014	0.61 ± 0.05**
Bafilomycin 3-month (43)	6.62 ± 0.56	6.90 ± 0.007	1.50 ± 0.10***
Bafilomycin 18-month (29)	11.38 ± 0.95	6.90 ± 0.008	0.75 ± 0.06**

*$P < 0.05$ vs. control 3-month-old; **$P < 0.05$ vs. control 18-month-old; ***$P < 0.05$ vs. EIPA 3-month-old.

proximal convoluted tubule acidification in old compared with young adult rats.

Results

Micropuncture experiments

Table 1 shows the acidification half times, which is the quotient between ln2 and the acidification rate constant, the luminal steady state pH (ssPH) and the bicarbonate flux ($J_{HCO_3^-}$). As indicated in Experimental procedures, this last value is calculated from the initial luminal perfusate, minus steady state bicarbonate concentration, multiplied by the acidification rate constant. As can be observed in the Table 1, all treatments affected mostly the acidification half times. The $J_{HCO_3^-}$ of old rats was 30% lower than in young rats. EIPA (5-N-ethyl-n-isopropyl amiloride) reduced $J_{HCO_3^-}$ by 60% in 3-month- and 18-month-old rats, corresponding to a reduction of 1.26 nmol · cm^{-2} · s^{-1}, and 0.85 nmol · cm^{-2} · s^{-1}. The $J_{HCO_3^-}$ of young and old rats after EIPA treatment were similar. The drop in $J_{HCO_3^-}$ after bafilomycin treatment was similar in 3-month-old and in 18-month-old rats. Bafilomycin diminished $J_{HCO_3^-}$ 0.62 nmol · cm^{-2} · s^{-1} in young rats, corresponding to a 30% reduction and 0.71 nmol · cm^{-2} · s^{-1}, representing an approximately 50% reduction in old rats. The sum of the EIPA-sensitive plus the bafilomycin-sensitive bicarbonate reabsorption in young rats was 2.36 nmol · cm^{-2} · s^{-1}, 0.24 nmol · cm^{-2} · s^{-1} larger than the value measured in control 3-month-old rats (Fig. 1A). The sum of the EIPA-sensitive plus the bafilomycin-sensitive $J_{HCO_3^-}$ in old rats was 1.36 nmol · cm^{-2} · s^{-1}, a value 7% lower than the bicarbonate reabsorption observed in the control 18-month-old rats (Fig. 1B). The magnitude of the bafilomycin-sensitive and the EIPA-sensitive component to proximal convoluted tubule acidification was the same in old but not in young rats.

Western blot experiments

Protein corresponding to the NHE3 isoform was detected in both young and old BBMV with a monoclonal antibody. Quantification of expression was performed using vesicles obtained from four separate samples of kidneys each taken from three rats. The purity of brush border membrane fraction was assessed by measuring the activity of γ-glutamyl transferase (Orlowsky & Meister, 1965) and the activity of Na^+-K^+ ATPase (Pecci *et al.*, 1994) in the homogenate and vesicle pellet. Na^+-K^+ ATPase activity was not detectable, but the activity of γ-glutamyl transferase increased 10-fold in both groups compared with the original homogenate.

The amount of the Na^+/H^+ exchanger was the same in aged rats compared with young rats (Fig. 2). The abundance of the

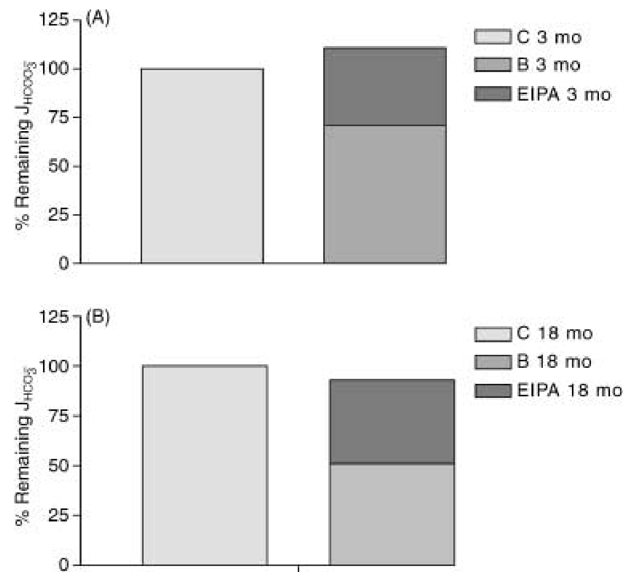

Fig. 1 The figure shows the sum percentage of the bicarbonate flux ($J_{HCO_3^-}$) remaining after treatment with bafilomycin (10^{-6} M) (B) and with EIPA (10^{-4} M) (EIPA) in young (3-month) (A) and old (18-month) (B) rats compared with the value observed without treatment (C).

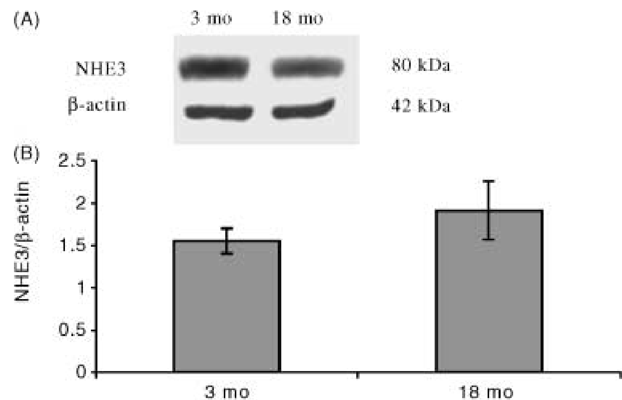

Fig. 2 (A) Western blot developed using an antibody against the NHE3 isoform of the Na^+/H^+ exchanger in vesicles of brush border membranes of proximal convoluted tubule from young (3-month) and old(18-month) rats. NHE3 is visualized at 80 kDa, while the band at 42 kDa is β-actin. (B) Densitometric measurements. Data are presented as the ratio of NHE3 to β-actin protein levels. Values are means ± SEM. Four Blots were made, each from a pool of tissues taken from three rats.

© 2006 The Authors
Journal compilation © Blackwell Publishing Ltd/Anatomical Society of Great Britain and Ireland 2006

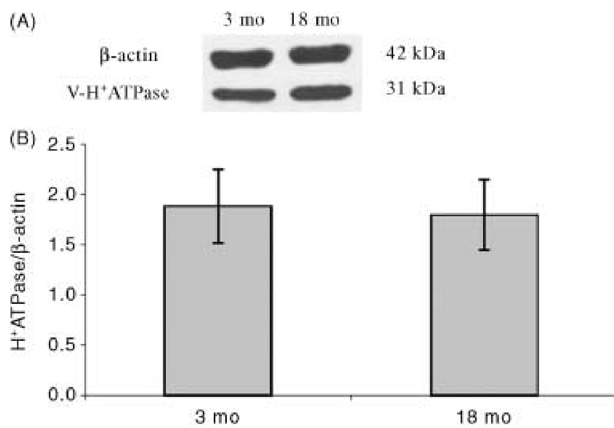

Fig. 3 (A) Western blot developed using an antibody against subunit E of the V-H^+ATPase in vesicles of brush border membranes of proximal convoluted tubule (BBMV) from young (3-month) and old (18-month) rats. The subunit E of the V-H^+ATPase is visualized at 31 kDa while the band at 42 kDa is β-actin. (B) Densitometric measurements. Data are presented as a ratio of V-H^+ATPase to β-actin protein levels. Values are means ± SEM. Four blots were made, each from a pool of tissues taken from three rats.

V-H^+ATPase was also quantified. The amount of protein was similar in both young and old rats (Fig. 3).

Discussion

In a previous study we found that aging is accompanied by a decrease in H-ion flux in BBMVs of proximal convoluted tubule (MacLaughlin *et al*., 2001). In the present study we quantify the participation of the EIPA- and bafilomycin-sensitive components in the total $J_{HCO_3^-}$ of young (3-month-old) and aging (18-month-old) rats. We used EIPA and bafilomycin as inhibitors of the NHE3 isoform of the Na^+/H^+ exchanger and the V-H^+ATPase, respectively. We confirmed previous results indicating that proximal tubule acidification is diminished in old rats (MacLaughlin *et al*., 2001). We also confirm that a large fraction (~60%) of bicarbonate reabsorption in the rat proximal tubule is dependent on the operation of an EIPA-sensitive mechanism (Preisig *et al*., 1987; Wang *et al*., 1999, 2001; Bailey, 2004). In agreement with others (Wang *et al*., 1999, 2001; Bailey, 2004), we found a significant component of bicarbonate transport mediated by the bafilomycin-sensitive V-H^+ATPase in the proximal tubules of both young and old rats.

The bicarbonate reabsorption in proximal convoluted tubules of 3-month-old rats, either with EIPA or with bafilomycin in the luminal solution, were, respectively, 60% and 30% lower than the $J_{HCO_3^-}$ measured under control conditions. The bafilomycin-sensitive acidification was significantly smaller than the EIPA-sensitive one, showing that normal acidification is carried out mostly by an EIPA-sensitive mechanism. These results concur with data on bicarbonate reabsorption obtained with a similar experimental approach (Bailey, 2004). In $NHE3^{-/-}$ mice, bicarbonate reabsorption is reduced 50–60% (Schultheis *et al*., 1998), a figure close to that reported in the present work. It is possible that the NHE3 isoform represents the main component affected by EIPA, since it has been reported that the NHE2 contribution to proximal acidification is negligible (Wang *et al*., 1999; Choi *et al*., 2000; Wang *et al*., 2001), although a Na^+-dependent EIPA-sensitive proton transport mechanism, different from the NHE2 and NHE3, in the proximal tubule has been proposed (Choi *et al*., 2000; Goyal *et al*., 2003).

There were no significant differences between the $J_{HCO_3^-}$ inhibited by bafilomycin and that inhibited by EIPA in aged rats, suggesting that both mechanisms contributed equally to the proximal bicarbonate reabsorption. Interestingly, the contribution of the vacuolar ATPase to proximal convoluted tubule acidification was larger in old rats than in young rats, suggesting that our observation could be in line with those of Baum (1992), who found a similar phenomenon in neonatal compared with adult rats.

The reduction of $J_{HCO_3^-}$ induced by bafilomycin was similar in old and young rats. This result is in agreement with the absence of differences in the abundance of the V-H^+ATPase between young and old rats. Thus, it is unlikely that the fall in the total acidification detected in old rats could be ascribed to the V-H^+ATPase. We were unable to detect a fall in NHE3 abundance; thus the present results could indicate that an EIPA-sensitive mechanism in addition to the NHE3 isoform is impaired in proximal tubule acidification of aging rats. It has been pointed out that NHE2 isoform does not contribute to proximal tubule acidification in mice lacking both NHE2 and NHE3 isoform (Wang *et al*., 1999; Choi *et al*., 2000). Goyal *et al*. (2003, 2005) described a new member of the family of mammalian NHE exchangers, the NHE8 isoform. This new isoform is a candidate to mediate Na^+-dependent acid extrusion across the apical membrane of proximal tubule cells (Choi *et al*., 2000). NHE8-mediated transport is retained in NHE3/NHE2 null mice and can be inhibited by EIPA (Choi *et al*., 2000). Thus, if NHE2 does not participate in proximal bicarbonate reabsorption in the proximal convoluted tubule of the rat, it is tempting to speculate that the EIPA-sensitive H-ion transport described by Choi *et al*. (2000) and Goyal *et al*. (2003, 2005) is also affected in old rats. However, it cannot be ruled out that the NHE3 isoform activity could also be involved. It is interesting that in old rats, as in the NHE3 null mice, the remaining HCO_3^- reabsorption is due to a bafilomycin-sensitive component, which does not up-regulate in spite of the severe reduction in the total amount of bicarbonate reabsorption (Wang *et al*., 1999). This defect does not appear to affect the whole animal acid-base equilibrium since there are no changes in blood acid-base status of old rats (MacLaughlin *et al*., 2001). However, during aging the capacity of the organism to regulate acid-base homeostasis is decreased (Syneok, 1976; Frassetto & Sebastian, 1996). In conclusion, proximal tubule acidification appears to be affected in aging by decreasing the activity of an EIPA-sensitive component without changes in the NHE3 abundance in the apical membrane.

Experimental procedures

Two groups of rats were studied, young adults (3-month-old) and aging rats (18-month-old).

© 2006 The Authors
Journal compilation © Blackwell Publishing Ltd/Anatomical Society of Great Britain and Ireland 2006

Micropuncture experiments

Rats were anesthetized with Inactin (100 mg kg^{-1} body weight i.p.), and were placed on a thermostatically controlled heated table and prepared by standard micropuncture techniques. The kinetics of acidification in proximal convoluted tubule were studied by continuous measurement of intratubular pH as previously described (Diaz-Sylvester *et al*., 2001). Briefly, the proximal convoluted tubule was perfused by means of a double-barrelled micropipette, one barrel filled with Sudan-Black-colored castor oil and the other with the perfusion solution (in mM: 75 NaCl, 5 KCl, 1 $CaCl_2$, 25 HCO_3, 1.25 $MgSO_4$, 10 glucose and 90 raffinose). The pH of the luminal solution was adjusted to 7.4, and the osmolality, measured with a vapor pressure osmometer (model 5100C, Wescor, Logan, UT, USA), was 290 mosmol kg^{-1}. Luminal pH changes were measured with an H^+-sensitive resin microelectrode (Fluka, Cocktail A, Ronkokoma, NY, USA) (Diaz-Sylvester *et al*., 2001). The slope of microelectrodes was 56 ± 2 mV/pH unit. Changes in luminal pH were measured in a buffer drop isolated between two castor oil columns. Because of the presence of raffinose, the drop of buffer does not change the volume and remains stationary; thus the change in pH is measured until it reaches the steady state.

The micropuncture experiments were performed under three conditions: (i) control, (ii) bafilomycin 10^{-6} M added to the luminal solution, and (iii) EIPA (5-N-ethyl-N-isopropyl amiloride; 10^{-4} M) added to the luminal solution.

Theoretical asumptions

During luminal perfusion, H^+ secretion results in acidification of the luminal solution and titration of alkaline buffer. Therefore, bicarbonate concentration falls and reaches steady state. Detailed treatments of this model have been previously published (Amorena *et al*., 1984). Using pH values recorded from microelectrode measurements, HCO_3^- concentration at time *t* is calculated according to:

$$[HCO_3^-] = 10^{(pH-pK)} \times \alpha \times pCO_2$$

Where $\alpha = 0.024$, pK = 6.03 (Edsall & Wyman, 1958) and pCO_2 = the partial pressure of CO_2 in mmHg. To calculate acidification rates, the log of ($[HCO_{3\infty}] - [HCO_{3t}]$), where $[HCO_{3\infty}]$ and $[HCO_{3t}]$ are the concentration of HCO_3^- at steady state and at time *t*, respectively, is plotted against time, in seconds. This plot can be fitted to a straight line, meaning that $[HCO_3^-]$ approaches exponentially to its steady-state value. The slope of this line is the acidification rate constant (κ). Net proton flux (J_H^+) is calculated according to:

$$J_H = [HCO_3^- - HCO_{3\,t=0}^-] \times k \times r/2$$

where *r* is the tubule radius in centimeters [0.0015 cm in young and 0.0019 cm in old rats (MacLaughlin *et al*., 2001)].

In vitro experiments

Brush border membrane vesicles

Brush border membrane vesicles from renal cortex were isolated in young and old rats using a technique previously described (Igarreta *et al*., 1996). Kidneys were removed, put in cold HEPES-sucrose-EDTA (HSE) buffer (in mM: 50 sucrose, 10 Tris, 10 HEPES and 0.5 EDTA, pH 7.5) and washed with the same buffer, decapsulated and renal cortex was separated. After differential centrifugation, the vesicle pellet was dissolved in HSE buffer with protease inhibitors (aprotinine 10 μg mL^{-1}, leupeptine 10 μg mL^{-1}, pepstatin A 10 μg mL^{-1}, phenylmethylsulfonyl fluoride 2 mM and dithiothreitol 1 mM). Protein concentration was determined according to Lowry *et al*. (1951).

Western blot

The abundance of Na^+/H^+ exchanger, isoform NHE3, and subunit E of the V-H^+ATPase in BBMV were assessed by Western blot analysis.

Evaluation of V-H+ATPase abundance. Brush border membrane vesicles corresponding to 100 μg protein were resuspended, heated at 100 °C for 2 min, separated electrophoretically on a 10% sodium dodecylsulfate polyacrylamide gel (SDS-PAGE) according to Laemmli (1970), transferred to a nitrocellulose membrane and blocked in Tris-Buffer-Sodium-Tween (TBST) (in mM: 20 Tris, 150 NaCl and 0.1% Tween 20, pH 7.5) with 5% nonfat milk for 1 h at room temperature with gentle agitation. The membrane was incubated with an antibody against subunit E of the V-H^+ATPase (rabbit polyclonal antibody, # sc-20946, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), diluted 1 : 500 in TBST, for 1 h at room temperature with gentle agitation. After five washes with TBST, the membrane was incubated with alkaline phosphatase-labeled secondary antibody (goat antirabbit IgG from Santa Cruz Biotechnology) dilution 1 : 5000. Bands were visualized with BCIP/NBT Color Development Substrate (Promega Corporation, Madison, WI, USA).

Quantification of NHE3. The general procedure is similar to that described above for quantification of V-H^+ATPase, with the following modifications: BBMV samples corresponding to 40 μg protein were electrophoresed on 8% SDS-PAGE. Transblots were incubated with the primary antibody (MAB against isoform NHE3, # MAB3138, Chemicon International, Temecula, CA, USA) diluted 1 : 500 in TBST, for 1 h at room temperature with gentle agitation. After five washes with TBST, the membrane was incubated with the secondary antibody (horseradish peroxidase-conjugated rat antimouse IgG_1 monoclonal antibody, # 559626 from BD Biosciences, San Jose, CA, USA) dilution 1 : 500. Bands were visualized with chemiluminescence detection reagents (ECL, Amersham, Piscataway, NJ, USA) and blue-light sensitive autoradiography film (Hyperfilm, Bio-Rad, Hercules, CA, USA).

Membranes containing the same samples in all cases were incubated with the primary antibody against β-actin at 1 : 5000 dilution (antibody against actin Ab5, # 612656 from BD

© 2006 The Authors
Journal compilation © Blackwell Publishing Ltd/Anatomical Society of Great Britain and Ireland 2006

Biosciences). After five washes with TBST, the membrane was incubated with the secondary antibody (antimouse IgG (H+L) alkaline phosphatase conjugate, # S372B, Promega Corporation).

Band intensities for both V-H^+ATPase and NHE3 isoform were determined by densitometric analysis using the ImageJ program provided by NIH website (http://molbio.info.nih.gov/molbio/Software.htm) and were normalized using the intensity of the β-actin band on the same blot (Dixit *et al*., 2004).

Experiments were repeated four times with protein samples drawn from different groups of young adult and aged rats.

Statistics

Results are expressed as means ± SE. Statistical analysis of data was performed by ANOVA and Newman–Keuls test. Western blot data were analyzed using Student's *t*-test. Differences were considered statistically significant at $P < 0.05$.

All the experiments have been conducted in conformity with the principles stated in American Physiological Society (APS)'s Guiding Principles in the Care and Use of Animals.

Acknowledgments

This work has been supported by the Agencia Nacional de Promoción Científica y Tecnológica (PICT: 05-8305) and by the Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 0851). The authors would like to thank Dr. A. A. Altamirano for critical reading of this manuscript and Dr. H. Schteingart and Lic. G. Mazzone, Centro de Investigaciones Endocrinológicas, Hospital de Niños 'R. Gutiérrez' for their contribution in the γ-glutamyl tranferase assay and S. Caram for technical assistance.

References

Amorena C, Castro AF (1997) Control of proximal tubule acidification by the endothelium of the peritubular capillaries. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **272**, R691–R694.

Amorena C, Fernandes D, Malnic G (1984) Factors affecting proximal tubular acidification of non-bicarbonate buffers. *J. Physiol. (Lond.)* **352**, 31–48.

Bailey MA (2004) Inhibition of bicarbonate reabsorption in the rat proximal tubule by activation of luminal $P2Y_1$ receptors. *Am. J. Physiol. Renal Physiol.* **287**, F789–F796.

Bank N, Aynedjain AS (1976) A micropuncture study of the effect of parathyroid hormone on renal bicarbonate reabsorption. *J. Clin. Invest.* **58**, 336–344.

Bank N, Aynedjian HS, Mutz BF (1985) Evidence for a DCCD-sensitive component of proximal bicarbonate reabsorption. *Am. J. Physiol.* **249**, F636–F644.

Baum M (1992) Developmental changes in rabbit juxtamedullary proximal convoluted tubule acidification. *Pediatr. Res.* **31** (4 Part 1), 411–414.

Baylis C (1994) Age dependent glomerular damage in the rat. *J. Clin. Invest.* **94**, 1823–1829.

Choi JY, Shah M, Lee MG, Schultheis PJ, Shull GE, Muallem S, Baum M (2000) Novel amiloride-sensitive sodium-dependent proton secretion in the mouse proximal convoluted tubule. *J. Clin. Invest.* **105**, 1141–1146.

Corman B, Pratz J, Poujeol P (1985) Changes in anatomy, glomerular filtration and solute excretion in old rat kidney. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **248**, R282–R287.

Davies DF, Shock NW (1950) Age changes in glomerular filtration rate, effective renal plasma flow and tubular excretory capacity in adult males. *J. Clin. Invest.* **29**, 496–507.

Diaz-Sylvester P, MacLaughlin M, Amorena C (2001) Peritubular fluid viscosity modulates H^+ flux in proximal tubules through NO release. *Am. J. Physiol. Renal Physiol.* **280**, F239–F243.

Dixit MP, Xu L, Xu H, Bai L, Collins JF, Ghishan FK (2004) Effect of angiotensin-II on renal Na^+/H^+ exchanger-NHE3 and NHE2. *Biochim. Biophys. Acta* **1664**, 38–44.

Edsall JT, Wyman J (1958) *Biophysical Chemistry*, Vol. I. New York: Academic Press, Inc.

Frassetto L, Sebastian A (1996) Age and systemic acid-base equilibrium: analysis of published data. *J. Gerontol. A Biol. Sci. Med. Sci.* **51**, B91–B99.

Goyal S, Mentone S, Aronson PS (2005) Immunolocalization of NHE8 in rat kidney. *Am. J. Physiol. Renal Physiol.* **288**, F530–F538.

Goyal S, Vanden Heuvel G, Aronson PS (2003) Renal expression of novel Na^+/H^+ exchanger isoform NHE8. *Am. J. Physiol. Renal Physiol.* **284**, F467–F473.

Hill L, Lateef AM, Engels K, Samsell L, Baylis C (1997) Basal and stimulated nitric oxide in control of kidney function in the aging rat. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **272**, R1747–R1753.

Igarreta P, Calvo JC, Paladini A, Damasco C (1996) Acute effects of two natural corticosteroids on the kinetics of the Na^+-H^+ exchanger in rat renal brush border membranes. *Can. J. Physiol. Pharmacol.* **75**, 1226–1231.

Jung FF, Kennefick TM, Ingelfinger JB, Vora JP, Anderson S (1995) Down-regulation of the intrarenal renin-angiotensin system in the aging rat. *J. Am. Soc. Nephrol.* **5**, 1573–1580.

Kinsella JL, Sacktor B (1987) Renal brush border Na^+-H^+ exchange activity in the aging rat. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **252**, R681–R686.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

Lindeman RD (1995) Renal and urinary tract function. In *Handbook of Physiology, Section 11: Aging* (Masoro EJ ed.). New York: Oxford University Press, pp. 485–503.

Lindeman RD, Tobin J, Shock NW (1985) Longitudinal studies on the rate of decline in renal function with age. *J. Am. Geriatr. Soc.* **33**, 278–285.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurements with the Folin phenol reagents. *J. Biol. Chem.* **193**, 265–275.

MacLaughlin M, Damasco MC, Igarreta P, Amorena C (2001) In vitro and in vivo evaluation of proximal tubular acidification in aging rats. *Am. J. Physiol.* **280**, R1627–R1631.

Manning RD, Hu L, Reckelhoff JF (1997) Role of nitric oxide in the arterial pressure and renal adaptations to long-term changes in sodium intake. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **272**, R1162–R1169.

Nakhoul NL, Hamm LL (2002) Vacuolar H^+-ATPase in the kidney. *J. Nephrol.* **15**, S22–S31.

Nelson N, Harvey WR (1999) Vacuolar and plasma membrane proton-adenosinetriphosphatases. *Physiol. Rev.* **79**, 361–385.

Orlowsky M, Meister A (1965) Isolation of γ-glutamyl transpeptidase from hog kidney. *J. Biol. Chem.* **240**, 338–347.

Papper S (1973) The effects of age in reducing renal function. *Geriatrics* **28**, 83–87.

Pecci A, Cozza EN, Devlin M, Gomez-Sanchez CE, Gomez-Sanchez EP (1994) Endothelin-1 stimulation of aldosterona and zona glomerulosa ouabain sensitive sodium/potassium ATPase. *J. Steroid Biochem. Mol. Biol.* **50**, 49–53.

Preisig PA, Ives HE, Cragoe EJ Jr, Alpern RJ, Rector FC Jr (1987) Role of the Na^+/H^+ antiporter in rat proximal tubule bicarbonate absorption. *J. Clin. Invest.* **80**, 970–978.

© 2006 The Authors
Journal compilation © Blackwell Publishing Ltd/Anatomical Society of Great Britain and Ireland 2006

Reilly AM, Harris PJ, Williams DA (1995) Biphasic effect of angiotensin II on intracellular sodium concentration in rat proximal tubules. *Am. J. Physiol.* **269**, F374–F380.

Schultheis PJ, Clarke LL, Meneton P, Miller ML, Soleimani M, Gawenis LR, Riddle TM, Duffy JJ, Doetschman T, Wang T, Giebisch G, Aronson PS, Lorenz JN, Shull GE (1998) Renal and intestinal absorptive defects in mice lacking the NHE3 Na^+/H^+ exchanger. *Nat. Genet.* **19**, 282–285.

Syneok LL (1976) Peculiarities of acid-base reserves in aging. *Ukr. Biokhim. Zh.* **48**, 72–77.

Wang T (1997) Nitric oxide regulates HCO_3^- and Na^+ transport by a cGMP-mediated mechanism in the kidney proximal tubule. *Am. J. Physiol. Renal Physiol.* **272**, F242–F248.

Wang T, Hropot M, Aronson PS, Giebisch G (2001) Role of NHE isoforms in mediating bicarbonate reabsorption along the nephron. *Am. J. Physiol.* **281**, F1117–F1122.

Wang T, Yang CL, Abbiati T, Schultheis PJ, Shull GE, Giebisch G, Aronson PS (1999) Mechanism of proximal tubule bicarbonate absorption in NHE3 null mice. *Am. J. Physiol.* **277**, F298–F302.

© 2006 The Authors
Journal compilation © Blackwell Publishing Ltd/Anatomical Society of Great Britain and Ireland 2006