In vitro and in vivo evaluation of proximal tubular acidification in aging rats

MYRIAM MAC LAUGHLIN,1 MARÍA CRISTINA DAMASCO,2 PILAR IGARRETA,1 AND CARLOS AMORENA1,3
1Instituto de Investigaciones Cardiológicas, Facultad de Medicina, 1122 Buenos Aires; 2Programa de Regulación Hormonal y Metabólica, Consejo Nacional de Investigaciones Centíficas y Técnicas, Departamento de Química Biológica, Consejo Nacional de Investigaciones Centíficas y Técnicas, Universidad de Buenos Aires, Buenos Aires; and 3Escuela de Ciencia y Tecnología, Universidad Nacional de Gral San Martín, 1650 San Martín, Argentina

Received 11 October 2000; accepted in final form 24 January 2001

Mac Laughlin, Myriam, María Cristina Damasco, Pilar Igarreta, and Carlos Amorena. In vitro and in vivo evaluation of proximal tubular acidification in aging rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R1627–R1631, 2001.—The normal aging process is accompanied by a progressive deterioration of renal function. We studied the kinetics of proximal tubular acidification of young (3 mo) and aging (22 mo) rats using in vivo and in vitro techniques. Blood acid-base parameters were similar in both groups. The maximum velocity of the Na+/H+ exchange (NHE) in brush-border membrane vesicles (BBMV) showed a 72% decrease in aging compared with young rats, whereas the Michaelis constant remained unchanged. The NHE3 isoform of the Na+/H+ exchanger was detected in BBMV by Western blot in both groups, and a decrease of 90% in the abundance was observed in aging rats. Micropuncture experiments with simultaneous luminal and peritubular perfusion with phosphate Ringer and continuous measurement of intratubular pH showed an acidification rate constant 34% smaller in aging compared with young rats. Proton flux was 48% lower in aging than in young rats. The present results suggest that proximal tubular acidification is impaired with aging.

Na+/H+; vesicles; micropuncture

AS PART OF THE NORMAL AGING PROCESS, the kidney develops a progressive deterioration of several structures and functions (7, 9). Glomerular filtration rate, renal blood flow, and concentrating ability decrease with age (8, 21, 26, 29). Aging also affects tubular function, although the mechanisms affected are less well defined (20). Under normal conditions, Na+/H+ exchange (NHE) accounts for about 65% of the proximal tubular acidification (18). The control of the exchanger is very complex and depends on many factors including, among others, the renin-angiotensin system (RAS) (30), endothelium-derived relaxing factor (EDRF) (2, 25, 32), and parathyroid hormone (6). There is down-regulation of the renal RAS with age, affecting renin mRNA and angiotensin-converting enzyme (17). In addition, renal hemodynamics of senescent rats seem to be more dependent on the EDRF than in younger animals (13). Kinsella and Sacktor (19) found a decrease of NHE activity in brush-border membrane vesicles (BBMV) from the renal cortex of kidneys from senile animals. On the other hand, Ikuma et al. (15) detected a decrease in the activity of the NHE in jejunal villus cells from senescent rats.

In the present work using in vivo and in vitro techniques, we studied the kinetics of proximal tubular acidification of aged rats. We performed micropuncture experiments with simultaneous luminal and peritubular perfusion, thus avoiding the effect of extratubular factors, and we evaluated the kinetics of the NHE of BBMV from the same population of rats. Our results suggest that proximal tubular acidification capacity in the aging rats is impaired.

METHODS

Groups of Rats

Two groups of male Wistar rats were used: young (3 mo) and aging (22 mo). They were allowed ad libitum access to standard laboratory rat chow and tap water.

Blood Acid-Base Status

A blood sample was taken from the tail in awake rats into heparinized capillary tubes without exposure to air. Acid-base parameters were measured in a Radiometer gas analyzer model ABL 330.

In Vitro Experiments

BBMV. BBMV from the renal cortex were isolated using a technique previously described (14). The vesicle pellet obtained after differential centrifugation was dissolved in HEPES-sucrose-EDTA (HSE) buffer (50 mM sucrose, 10 mM Tris, 10 mM HEPES, 0.5 mM EDTA, pH 7.5). Protein concentration was determined according to Lowry et al. (23). The purity of the brush border membrane fraction was assessed.
measuring the activity of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase (27) and of glutamyl transferase in the vesicle pellet. Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity was not detectable, but the activity of glutamyl transferase increased 10-fold compared with the original homogenate. Vesicles were prepared freshly from four animals for each experiment.

**NHE kinetics.** Transport was measured fluorometrically according to Igarreta et al. (14). Vesicles dissolved in HSE were loaded up to a final concentration of 150 mM Na-glucuronate at least 90 min before kinetic studies. Briefly, 20 μl of the vesicle preparation were diluted into 2 ml of external solution (in mM: 75 NaCl, 5 KCl, 1 CaCl\textsubscript{2}, 20 H\textsubscript{2}NaPO\textsubscript{4}, 1.25 MgSO\textsubscript{4}, and 10 glucose, pH 7.4). A fluid droplet of phosphate-Ringer buffer was injected between oil columns in the tubule lumen, and luminal pH changes were measured with an H\textsuperscript{+}-sensitive resin microelectrode (5) (Fluka, Cocktail A). The difference between [H\textsubscript{2}NaPO\textsubscript{4}]	extsubscript{a} at steady state (ss) and [H\textsubscript{2}NaPO\textsubscript{4}]	extsubscript{t} at time \( t \) diminishes exponentially with an acidification rate constant (\( k \)) (11). Net H\textsuperscript{+} secretion (\( J_{\text{H}^+} \)) was calculated as: \( J_{\text{H}^+} = ([H\textsubscript{2}NaPO\textsubscript{4}]	extsubscript{t} - [H\textsubscript{2}NaPO\textsubscript{4}]	extsubscript{a}) \times k \times (r^2) \), where \( r \) is the lumen radius of the tubule (15 μm in control and 19 μm in the aging rats), and [H\textsubscript{2}NaPO\textsubscript{4}]	extsubscript{t} and [H\textsubscript{2}NaPO\textsubscript{4}]	extsubscript{a} are the concentration of the injected phosphate at time \( 0 \) and at steady state, respectively. Microelectrodes were calibrated in phosphate-Ringer buffer pH 7 and 8, at the beginning and at the end of every group of acidification curves. The slope of microelectrodes was 56 ± 2 mV/pH unit.

**Statistics**

Results are expressed as means ± SE. Statistical analysis of data was performed by Student’s \( t \)-test.

**RESULTS**

**Acid-Base Parameters**

Blood pH, P\textsubscript{CO\textsubscript{2}}, and plasma bicarbonate concentration ([HCO\textsubscript{3}])\textsubscript{p} of young rats (\( n = 6 \)) were 7.36 ± 0.02, 42.6 ± 0.60 mmHg, and 23.3 ± 0.51 meq/l, respectively. In the aging rats (\( n = 6 \)), pH was 7.35 ± 0.02, P\textsubscript{CO\textsubscript{2}} was 43.5 ± 0.42 mmHg, and [HCO\textsubscript{3}])\textsubscript{p} was 23.2 ± 0.34 meq/l.

**NHE Kinetics**

We examined the kinetics of the NHE in vesicles submitted to an Na\textsuperscript{+} gradient. Data collected were fitted to the Michaelis Menten equation. The \( V_{\text{max}} \) was significantly reduced in aging rats [4,977 ± 264 fluorescence units (FU)/min, \( n = 3 \)] compared with young rats (27,672 ± 2,769 FU/min, \( n = 4 \)), whereas the \( K_m \) was unchanged (Table 1).

**Western Blot**

The result of a representative Western blot is shown in Fig. 1. Densitometry readings revealed a band of ~80 kDa that corresponds to the NHE3 isoform of the NHE with 3,593 arbitrary densitometry units (ADU) in young rats and with 281 ADU in aging rats. These results qualitatively agree with the in vitro transport experiments reported above.

**Micropuncture Experiments**

Table 2 and Fig. 2 show results obtained with the micropuncture experiments. The acidification rate constants for Na\textsuperscript{+}-H\textsuperscript{+} brush border membrane vesicles exchanger kinetics of young and aging rats are provided in Table 1. Values of Na\textsuperscript{+}-H\textsuperscript{+} brush border membrane vesicles exchanger kinetics of young and aging rats

<table>
<thead>
<tr>
<th></th>
<th>( V_{\text{max}}, \text{FU/min} )</th>
<th>( K_m, \text{mM} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>27,672 ± 2,769</td>
<td>27.40 ± 8.20</td>
</tr>
<tr>
<td>Aging</td>
<td>4,977 ± 264\textsuperscript{*}</td>
<td>15.34 ± 1.90</td>
</tr>
</tbody>
</table>

Values are means ± SE. Each experiment involved pooled vesicles from 4 animals. \( *P < 0.05 \) vs. young rats (Student’s \( t \)-test). \( V_{\text{max}} \), maximal velocity; \( K_m \), Michaelis constant; FU, fluorimetric units. Number of experiments in parentheses.
Acidification halftime calculated as \(\ln 2/k\) were 4.48 ± 0.43 s \((n = 15)\) in young and 6.36 ± 0.42 s \((n = 21)\) in aging rats \((P < 0.05)\). Luminal steady-state phosphate concentration \([H_2NaPO_4]_{ss}\) and pH \((pH_{ss})\) were the same in both groups of rats (Table 2). Luminal proton flux \((J_{H^+})\) calculated from \(k\) and \([H_2NaPO_4]_{ss}\) was 0.59 ± 0.086 nmol·cm\(^{-2}\)·s\(^{-1}\) in aging and 1.12 ± 0.097 nmol·cm\(^{-2}\)·s\(^{-1}\) in control young rats (Fig. 2) \((P < 0.05)\).

**DISCUSSION**

The present work shows that aging impairs proximal tubular acidification. This impairment is probably due to a decrease in the activity and abundance of brush border NHE. However, this defect did not affect blood acid-base status because blood pH, PCO\(_2\), and \([HCO_3^-]_p\) were normal. Our results agree with those of Prasad et al. (28), who did not detect blood pH differences between 6-mo and 24-mo-old rats. Frassetto et al. (10) showed that acid-base status of the blood changes in adult humans. From young adulthood to old age, men and women develop a low grade metabolic acidosis attributed by the authors to the age-related renal insufficiency (10). We have evaluated the acid-base status of a small group of six young and six senile anesthetized rats, whereas Frassetto et al. (10) performed a more extensive study designed to detect small changes in acid-base composition of 64 individuals, ranging from 17 to 74 yr old. Thus the lack of evidence of alterations of the acid-base status in aging rats could be due to the experimental design. Another factor that could contribute to the difference observed between aging humans and rats is the levels of insulin-like growth factor 1 (IGF-1) and growth hormones. IGF-1, a growth factor that stimulates apical NHE in proximal tubules (16), declines in aging humans but does not decline in aging rats (12, 31). The absence of acid-base defects in basal conditions does not exclude a defect in \([HCO_3^-]_p\) handling by the kidney of the aging rats, which would be manifest after an acid load (28).

In agreement with Kinsella and Sacktor (19), we found that both the amount of the NHE3 isoform measured by densitometry after isolation by Western blot and the kinetics of the NHE in BBMV were conspicuously diminished in 22-mo-old rats. A direct approach to evaluate the capacity of acidification of the proximal tubule is by using micropuncture techniques. These results are independent of glomerular filtration rate or any other hemodynamic or systemic variable, because luminal and peritubular perfusion is simultaneously performed. We found that aging rats had a significant decrease in the capacity of proximal tubular acidification, mostly due to a reduction of the acidification rate constant. Thus aging rats would have an intrinsic defect in the proximal tubular acidification.

We found a larger reduction of the NHE activity in vitro than in PCT acidification parameters measured in vivo. In the proximal tubule, NHE accounts for ~65% of apical membrane proton secretion, and ~35%

---

**Table 2. Values of \(k\), \([H_2NaPO_4]_{ss}\), and \(pH_{ss}\) of young and aging rats**

<table>
<thead>
<tr>
<th></th>
<th>(k), s(^{-1})</th>
<th>([H_2NaPO_4]_{ss}), meq/l</th>
<th>(pH_{ss})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young (15)</td>
<td>0.18 ± 0.02</td>
<td>12.31 ± 0.71</td>
<td>6.55 ± 0.06</td>
</tr>
<tr>
<td>Aging (21)</td>
<td>0.12 ± 0.01*</td>
<td>10.58 ± 0.80</td>
<td>6.74 ± 0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE. \(*\) Number of observations. \(*P < 0.05\) vs. young rats (Student’s \(t\)-test). \(k\), acidification rate constant; \([H_2NaPO_4]_{ss}\), steady-state phosphate; \(pH_{ss}\), steady-state pH.
is mediated by an H\(^+\)-ATPase (18). The difference between in vitro and in vivo observations suggests that an alternative mechanism(s) of proximal tubule acidification would take over the fall of the NHE activity. A possible mechanism involved could be an increase in the activity of H\(^+\)-ATPase compensating, in part, by the impairment in the NHE activity. On the other hand, because several paracrine and autocrine systems regulate the activity of the NHE in the proximal tubule (2, 6, 25, 30, 32), it is possible that part of the difference between in vivo and in vitro observations results from the participation of regulatory mechanisms present in the intact whole cell. This could be in accordance with results obtained by Ikuma et al. (15) studying the effect of aging on intracellular pH (pH\(_i\)) regulation in jejunal villus cells. They found that after inducing cytoplasmic acidification, the relationship between pH\(_i\) and external Na\(^+\) concentration showed a V\(_{\text{max}}\) of alkalization that was only 20% lower in senescent than in young rats. It is important to point out that the NHE3 isoform, responsible for NHE in the apical membrane of PCT, is also present in the brush border of small intestine epithelial cells (33). Moreover, Lorenz et al. (22) found a 38% decrease in proximal fluid reabsorption in homozygous NHE3/– knockout mice compared with the wild type. These results indicate that an important fraction of Na and volume reabsorption in the PCT is independent of the NHE at least under conditions where the exchange is absent or poorly expressed. Our results suggest that despite the large reduction in the NHE expression and activity, as determined by in vitro experiments and Western blot, bicarbonate claim in PCT of aging rats would not be reduced to the same magnitude.

In conclusion, aging rats showed an impaired PCT acidification capacity, probably as a result of a decrease in the activity and abundance of the NHE. However, the quantitative difference between in vivo and in vitro results could indicate the presence of a compensatory regulatory mechanism(s) acting in the proximal cell in vivo or an increase in the activity of other acidifying mechanisms present in the PCT, as in the H\(^+\)-ATPase.

**Perspectives**

This work shows, from our point of view, the importance of considering that the function of a whole system does not necessarily emerge from the activity of a single mechanism. Indeed, the clear reduction of the main component of PCT acidification in vitro, which is not accompanied by a similar fall in in vivo PCT acidification, indicates that the function of the organ is preserved independently of the failure of a single mechanism. This is strongly supported by data from Lorenz et al. (22) that demonstrate that the knockout of the NHE does not affect PCT function in the magnitude expected, despite the fact that under normal conditions, it accounts for most of the Na\(^+\) reabsorption along this segment. It seems that the NHE reduction in aging rats is partially compensated by other mechanism(s) to preserve acid-base balance. Nevertheless, this equilibrium is close to being unstable as an acidic load disrupts it (16). In conclusion, the acid-base status is apparently preserved by the takeover of mechanisms that are not fully operative in younger animals. It would be very important to identify those mechanisms, not only for a better comprehension of the aging process, but also for knowledge of basic kidney physiology.

We thank Drs. C. Jatimashiansky and S. Francioni from Hospital de Clínicas General José de San Martín for the determination of blood acid-base parameters, M. Zallocci for technical assistance, and Dr. A. Altamirano for critical reading of the manuscript. This work was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas, (#4606, 0521, and 6143).

**REFERENCES**


17. Jung FF, Kennefick TM, Ingelfinger JB, Vora JP, and Anderson S. Downregulation of the intrarenal renin-angioten-