Angiotensin II-mediated GFR decline in subtotal nephrectomy is due to acid retention associated with reduced GFR

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ABSTRACT

Background. Angiotensin II (AII) mediates glomerular filtration rate (GFR) decline in animals with subtotal nephrectomy (Nx), but the mechanisms for increased AII activity are unknown. Because reduced GFR of Nx is associated with acid (H⁺) retention that increases kidney AII, AII-mediated GFR decline might be induced by H⁺ retention.

Methods. We measured GFR and kidney microdialyzate H⁺ and AII content in Sham and 2/3 Nx rats in response to amelioration of H⁺ retention with dietary NaHCO₃, to AII receptor antagonism and to both.

Results. GFR was lower in Nx than that in Sham. Nx but not Sham GFR was lower at Week 24 than that at Week 1. Despite no differences in plasma acid-base parameters or urine net acid excretion, kidney H⁺ content was higher in Nx than that in Sham, consistent with H⁺ retention. Plasma and kidney microdialyzate AII were higher in Nx than that in Sham and dietary NaHCO₃ reduced each in Nx but not in Sham. AII receptor antagonism was associated with higher Week 24 GFR in Nx with H⁺ retention but not in Sham or in Nx in which H⁺ retention had been corrected with dietary NaHCO₃. Week 24 GFR after dietary NaHCO₃ was higher than after AII receptor antagonism. Week 24 GFR was not different after adding AII receptor antagonism to dietary NaHCO₃.

Conclusions. AII-mediated GFR decline in 2/3 Nx was induced by H⁺ retention and its amelioration with dietary HCO₃⁻ conserved GFR better than AII receptor antagonism in this CKD model. H⁺ retention might induce AII-mediated GFR decline in patients with reduced GFR, even without metabolic acidosis.

Keywords: aldosterone, angiotensin II, bicarbonate, chronic kidney disease, endothelin, microdialysis

INTRODUCTION

Animals with the 5/6 nephrectomy (Nx) model of chronic kidney disease (CKD) have progressive glomerular filtration rate (GFR) decline [1, 2] mediated through angiotensin II (AII) receptors [3]. Because anti-AII drug therapy helps conserve GFR in patients with reduced GFR [4, 5], AII appears to mediate GFR decline in CKD patients. AII also mediates increased nephron acidification in 5/6 Nx [6, 7] and 2/3 Nx [8]. Increased acidification in 2/3 Nx allows higher per nephron H⁺ excretion, thereby avoiding metabolic acidosis in this setting of reduced overall GFR [8]. So, further GFR decline in animals with reduced GFR might be a pathophysiologic consequence of kidney physiologic mechanisms to maintain steady-state H⁺ excretion in the face of unchanged dietary H⁺. Specifically, kidney AII induced by H⁺ retention in 2/3 Nx yields the short-term physiologic benefit of increased per nephron acidification but might also yield the longer-term pathophysiologic consequence of GFR decline through AII receptors. If so, reducing H⁺ retention with dietary HCO₃⁻ might lower kidney AII and thereby help conserve GFR. The present study tested the hypothesis that the progressive GFR decline of 2/3 Nx is mediated by H⁺ retention through AII receptors.

MATERIALS AND METHODS

Animals, diet and protocol

Eight-week-old male and female Munich-Wistar rats (Harlan Sprague-Dawley, Houston, TX) ate standard chow (Prolab RMH 2500 with 23% protein of various sources, Purina Labs, St Louis, MO) before kidney mass reduction surgery (below). Because daily NaHCO₃ slowed GFR decline in patients with CKD stage-2
eGFR without metabolic acidosis [9], we developed an animal model of such subjects. Unlike conscious 5/6 Nx that had lower arterial plasma total CO$_2$ (PTCO$_2$) than Sham [2] by ultrafluorometry [10], PTCO$_2$ by fluorometry was comparable in conscious 2/3 Nx and Sham despite greater kidney and skeletal muscle H$^+$ content in 2/3 Nx [8, 11, 12]. So, we used 2/3 Nx to determine whether H$^+$ retention mediated GFR decline and did so through AII.

After kidney mass reduction, animals ate minimum electrolyte diets with 20% protein as casein (ICN Nutritional Biochemicals, Cleveland, OH) and drank distilled H$_2$O. We compared GFR at 1 and 24 weeks after nephron mass reduction rather than 12 weeks [2] to allow for greater GFR decline. To examine AII as a potential mediator of GFR decline in 2/3 Nx with reduced GFR and H$^+$ retention but without metabolic acidosis, other animals received oral NaHCO$_3$ to reduce H$^+$ retention as discussed. We assessed H$^+$ retention with in vivo microdialysis of kidney cortex [11–13]. Like Sham, Nx eating diets with 150 μM NaCl/g diet/day of added NaHCO$_3$ had no net H$^+$ addition to microdialyzate [11] (see below). Previous studies [2] showed that H$^+$ retention in animals ingesting 150 μM NaCl diet/day of added NaCl was not different from baseline Nx. Because standard chow in our laboratory contained 150 μM NaCl/g diet, the dietary Na$^+$ load was comparable with that routinely received in our laboratory. Nx and similar weight controls ate 17.5 ± 0.9 versus 18.4 ± 0.9 g/day, respectively, (n = 4, P = 0.09) and so all received 17 g/day.

Because valsartan has greater affinity for the AT 1 receptor than losartan [13], some animals received valsartan (Novartis Pharmaceuticals, East Hanover, NJ), mixed with diet at 10 mg/kg body weight (bw)/day, a dose that reduced AII-induced aldosterone synthase mRNA in rats [14]. As detailed previously [8], we attempted to determine the maximum tolerated valsartan dose. Although animals tolerated valsartan as high as 25 mg/kg given alone, tail-cuff blood pressure was considerably lower (81.0 ± 5.0 versus 99.2 ± 5.9 n = 4, P = 0.11) when this valsartan dose was combined with darusentan and eplerenone as dictated by one protocol arm (see below). Importantly, GFR at Week 24 was not different between 2/3 Nx given 25 mg/kg bw/day compared with 10 mg/kg bw/day valsartan alone (2179 ± 209 versus 2150 ± 202 μL/min, respectively, n = 4, P = 0.86), supporting that 10 mg/kg bw/day yielded maximum GFR preservation. Because earlier studies showed that GFR decline in 2/3 Nx was mediated in part through endothelin A and aldosterone receptors [12], some animals received valsartan along with the endothelin A receptor antagonist darusentan (Knoll, AG, Ludwigshafen/Rhein, Germany) mixed with diet at 20 mg/kg bw/day and the aldosterone antagonist eplerenone (Pfizer, New York, NY) in diet at 100 mg/kg bw/day to determine whether these two receptor antagonist provided additional GFR conservation to valsartan.

Kidney mass reduction

Nx was induced by surgical removal of ~2/3 of kidney mass in two stages and chronic vascular lines inserted [11]. One week after the second surgery during which animals ate the described experimental diet, GFR was measured in conscious Nx and Sham by slope of the decrease in plasma concentration of intravenously infused $^3$H-inulin over 180 min [15].

**Microdialysis to compare kidney cortical H$^+$ and AII**

Twenty-three weeks after the second surgery during which animals ate the experimental diet, a microdialysis catheter was inserted into the kidney cortex of Nx and Sham through a flank incision [11, 12]. Relative H$^+$ retention was determined by comparing H$^+$ content ([H$^+$] times dialyzate volume) between collected and infused dialyzate [11, 12]. Kidney cortical AII content was similarly compared as done previously [8].

Microdialysis of kidney cortex was done in comfortably restrained, conscious animals 7 days after microdialysis catheter insertion (24 weeks after kidney mass reduction) using a dialyzate that would gain H$^+$ when dialyzed against kidney cortex with higher-than-Sham H$^+$ content and would lose H$^+$ if kidney cortex H$^+$ were less [11, 12]. Three 20-min collection periods were done in eight animals in each group. Anaerobically obtained, collected and infused dialyzates were analyzed for pH (Micro flow through pH monitor, Lazar Research Labs, Los Angeles, CA), PCO$_2$ (Micro flow through CO$_2$ probe, Lazar Research Labs) and total CO$_2$ (TCO$_2$) by flow-through ultrafluorometry [10].

**Whole blood and plasma parameters**

Immediately after microdialysis (24 weeks after kidney mass reduction), 0.35 mL of carotid arterial blood was slowly removed for arterial blood gases, plasma total CO$_2$ (by flow-through ultrafluorometry), electrolytes and AII from awake, calm and gently restrained animals. It was replaced with an equivalent blood volume from a paired, identically treated animal. The animal was then returned to its metabolic cage for an additional 1 week. GFR was repeated, now 24 weeks after initial GFR measurement and 25 weeks after kidney mass reduction. Additional studies were done in two groups of separate animals to determine the effect of valsartan discontinuation on GFR. In Group 1, valsartan was discontinued for the entire 24 weeks as described. In Group 2, valsartan was stopped at Week 23. In both groups, GFR was measured at Weeks 1, 23 and 24.

**Urine NAE**

Six days after insertion of the microdialysis catheter, urine NAE was measured [16] in a 24-h sample in eight animals each of control and experimental groups kept in metabolic cages.

**Analytical methods**

Dialyzate and plasma AII were measured as described previously [8] with an RIA kit (SPI-BIO, France) after storage at –80°C in tubes containing EDTA, Pepstatin, enalaprilate and 1,10-phenanthroline and evaporated to dryness, as described previously [17].

**Calculations**

Urine NAE was the mean for each animal group. Net dialyzate H$^+$ addition was averaged for the three collection periods per animal that was then averaged for each animal in each group [11, 12]. Positive values for net H$^+$ addition...
indicated greater H⁺ content in collected dialyzate compared with infused dialyzate (i.e. H⁺ gain) and negative ones indicated lower H⁺ content (i.e. H⁺ loss).

**Statistical analysis**

Continuous variables were first examined for normality and non-parametric tests such as a Kruskal–Willis test for more than two groups and a Wilcoxon ranks-sum test for paired samples were considered. The changes of GFR from pre to post for each group were described by mean and SD and they were considered with a one-sample t-test. The differences among treatments were considered with one-way ANOVA followed by post hoc Tukey’s test. Data management and statistical analysis were performed using SAS software (version 9.3, SAS Institute, Cary, NC) and graphs were created using R 3.0 (R Core Development Team, 2013). A P-value of <0.05 indicates a statistical significance.

**RESULTS**

Plasma acid–base parameters and K⁺ were not different between Nx and Sham but Nx had higher tail-cuff blood pressure as in Table 1. Microdialyzate H⁺ addition was higher in Nx than that in Sham, consistent with H⁺ retention in Nx. Also, Nx had higher plasma AII and higher microdialyzate AII. Nx and Sham urine net acid excretion (UA(NAE)) was not different but Nx had lower urine excretion of NH₄⁺ and HCO₃⁻.

Table 2 shows the effects of oral valsartan (Val), oral NaHCO₃ (HCO₃⁻) and their combination (Val + HCO₃⁻), on the indicated parameters within treatment groups (*) and for treatments within Nx and Sham across the three treatment groups (**). Table 3 shows the intra-group comparisons for the data given in Table 2. With the exception of higher plasma and microdialyzate AII in treated animals, valsartan and NaHCO₃ did not affect parameters, including microdialyzate H⁺, in Sham. In contrast, plasma pH, PCO₂, total CO₂ and NaHCO₃ addition were higher in Sham than that in Nx. Tail-cuff blood pressure was lower in Nx given valsartan than that in baseline (P < 0.01) and blood pressure was higher in Nx given NaHCO₃ than that in baseline and valsartan-treated Nx (P < 0.01). Blood pressure in Nx given valsartan and NaHCO₃ was lower than those given NaHCO₃ alone (P < 0.03).

Figure 1 shows that GFR was lower in Nx than in Sham at both time points (Weeks 1 and 24) as expected. Although Sham GFR was not different between Week 24 and Week 1 (1408 ± 163 versus 4139 ± 269 µL/min, respectively, P = 0.42), GFR in Nx was lower at Week 24 than that at Week 1 (1700 ± 159 versus 2562 ± 143 µL/min, respectively, P < 0.01). Figure 2 shows that neither valsartan nor NaHCO₃ affected Week 24 GFR in Sham. In contrast, Figure 3 shows that Nx had lower GFR at Week 24 compared with Week 1 for baseline (1644 ± 135 versus 2526 ± 80 µL/min, respectively, P < 0.01), after valsartan (2128 ± 84 versus 2515 ± 119 µL/min, respectively, P < 0.01) and after NaHCO₃ (2349 ± 152 versus 2542 ± 143 µL/min, respectively, P < 0.01). Consequently, valsartan and NaHCO₃ helped conserve GFR in Nx with H⁺ retention but had no GFR effect in Sham without H⁺ retention. In Figure 4, GFR at Week 24 of Nx given valsartan was higher than Nx GFR at Week 24 of baseline Nx (P < 0.01) and GFR at Week 24 of Nx given NaHCO₃ was higher than Nx GFR at Week 24 of Nx given valsartan (P < 0.01).

Studies in Figure 4 determined whether valsartan additionally preserved GFR in Nx given NaHCO₃. GFR was lower at Week 24 than Week 1 in Nx given valsartan (2153 ± 115 versus 2526 ± 80 µL/min, respectively, P < 0.01), in Nx given NaHCO₃ (2333 ± 119 versus 2537 ± 94 µL/min, respectively, P < 0.01) and in Nx given valsartan + NaHCO₃ (2308 ± 80 versus 2530 ± 82 µL/min, respectively, P < 0.01). GFR at Week 24 of Nx given NaHCO₃ was higher than GFR of Nx given valsartan at Week 24 (P < 0.01) but GFR at Week 24 of Nx given valsartan + NaHCO₃ was no different than GFR of Nx given NaHCO₃ alone (P = 0.89).

Because GFR decline in 2/3 Nx was mediated through endothelin A and aldosterone receptors [12], studies in Figure 5 explored whether adding the endothelin A receptor antagonist darusentan and the aldosterone antagonist spironolactone provided GFR preservation additional to valsartan. Figure 5 shows lower GFR at Week 24 than that at Week 1 in Nx given NaHCO₃ (2352 ± 67 versus 2572 ± 60 µL/min, respectively,
P < 0.01, in Nx given valsartan (2155 ± 64 versus 2563 ± 63 µL/min, respectively, P < 0.01) and in Nx given the three drugs (2249 ± 60 versus 2550 ± 56 µL/min, respectively, P < 0.01).

GFR at Week 24 of Nx given valsartan and Nx given the three drugs were each lower than Nx given NaHCO3 (P < 0.02). In addition, GFR at Week 24 for Nx given the three drugs was higher than Nx given valsartan (P < 0.02).

Because in previous studies 2/3 Nx given HCO3 had better GFR conservation than those given darusentan and eplerenone combined and that these antagonists added no GFR preservation to oral HCO3 [12], studies in Figure 6 determined whether oral NaHCO3 provided GFR conservation in addition to that provided by the three antagonists given together. Figure 6 shows lower GFR at Week 24 than that at Week 1 in Nx given NaHCO3 (2415 ± 54 versus 2576 ± 53 µL/min, respectively, P < 0.01), in Nx given the three drugs (2287 ± 29 versus 2537 ± 67 µL/min, respectively, P < 0.01) and in Nx given the three drugs + NaHCO3 (2410 ± 62 versus 2541 ± 47 µL/min, respectively, P < 0.01). GFR at Week 24 of Nx given the three drugs was lower than Nx given NaHCO3 (P < 0.01) and lower than Nx given the three drugs + NaHCO3 (P < 0.01).

GFR was not different between Nx given NaHCO3 and those given the three drugs + NaHCO3 (P = 0.98).

Because anti-AII drugs might lower GFR in some patients with reduced GFR [5], we explored whether GFR increased in animals chronically taking valsartan after the drug was stopped. Table 4 shows GFR measurements of two groups of separate animals at Weeks 1, 23 and 24. Group 1 continued valsartan throughout the 24-week protocol. In contrast, Group 2 had valsartan stopped at Week 23 and GFR was measured 1 week later. GFR at Week 23 was lower than that at Week 1 for both groups. Week 24 GFR was not different from Week 23 GFR in Group 1 but Week 24 was higher than Week 23 GFR in Group 2 (P < 0.05, paired t).

Nevertheless, there was no difference in Week 24 GFRs between Groups 1 and 2.

**DISCUSSION**

The present study showed that AII receptor antagonism preserved GFR in Nx with H+ retention but did not do so in Sham that had no H+ retention nor in Nx in which H+...
retention had been corrected with NaHCO₃. Because AII receptor antagonism had no effect on H⁺ retention, its GFR preservation appeared not to be mediated through reduced H⁺ retention. In addition, Nx had higher than Sham microdialyzate AII that was reduced when H⁺ retention was corrected with NaHCO₃, supporting that amelioration of H⁺ retention with NaHCO₃ (but not with NaCl as in previous studies [12]) lowered kidney AII. Furthermore, NaHCO₃ did not affect follow-up GFR in Sham. These data affirm earlier studies showing that H⁺ retention mediates GFR decline in the 2/3 Nx model of CKD [12] and show, for the first time, that this detrimental effect of H⁺ retention on GFR is mediated through AII receptors.

Adding endothelin A and aldosterone receptor antagonism to AII receptor antagonism preserved GFR better than AII receptor antagonism alone, supporting additive roles for endothelin and aldosterone in the GFR decline of 2/3 Nx [12]. Because AII stimulates kidney production of endothelin [18] and aldosterone [19], AII receptor antagonism alone might inhibit kidney endothelin and/or aldosterone secretion and thereby inhibit contributions of these two agents to GFR decline. Nevertheless, increased extracellular H⁺ stimulates secretion of endothelin from kidney microvascular endothelial cells [20] and of aldosterone from adrenal cells [21]. Consequently, H⁺ retention in 2/3 Nx might increase kidney levels of endothelin and aldosterone independent of AII and therefore might explain why endothelin A and aldosterone receptor antagonism provided added GFR conservation to AII receptor antagonism.

The present study shows a small but significant GFR increase 1 week after valsartan that had been administered for 23 of the 24-week protocol was discontinued. These data are consistent with the small hemodynamic effect of anti-AII drugs to decrease GFR in patients [5]. Nevertheless, the 24-week GFR was not different between animals in which valsartan had been stopped for 1 week compared with animals in which valsartan was continued for 24 weeks. These data support that any anti-AII effect of valsartan to decrease GFR was small and that the 24-week GFR of animals that continued valsartan for

Table 3. Multiple intra-group comparisons

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<th>HCO₃ versus</th>
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<td>Nx</td>
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FIGURE 1: Box plots comparing whole-kidney GFR (µL/min) of animals with 2/3 surgical nephron mass reduction (Nx, n = 8) with sham-operated animals (Sham, n = 8), Week 1 and Week 24 after surgery. The dark horizontal line represents the median and the box encompasses values within the 25th percentile above and the 25th below the median. *P < 0.05 versus respective Week 1 value; +P < 0.05 versus respective Sham.

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24 weeks is reflective of the degree of GFR conservation induced by valsartan and that there is no greater GFR conservation that is being concealed by such a hemodynamic effect.

Pharmacologic kidney protection for patients with reduced GFR is currently done with antagonists of AII and aldosterone, and endothelin antagonists are being explored as kidney protectors.
blood pressure was much higher in 5/6Nx given NaHCO₃. Furthermore, amelioration of H⁺ retention with oral NaHCO₃ likely salt to be used for dietary acid reduction [24] and shows better tolerated, less expensive and possibly more effective than other investigators using microdialysis report that the microdialyzate interfaces with interstitial fluid [33]. We hypothesize that interstitial fluid is at least one compartment containing the excess free H⁺. With comparatively little protein, H⁺ additions to interstitial fluid would cause rapid and large [H⁺] changes compared with plasma because of its relative lack of buffers. We further hypothesize that these measured [H⁺] changes signal, among other kidney responses, increased kidney levels of AII, endothelin A and aldosterone receptors. In addition, combined three-receptor antagonism provided no additional GFR preservation when added to NaHCO₃. Together, the present and previous studies support that H⁺ retention mediates GFR decline in 2/3Nx in part through AII, endothelin and aldosterone receptors but that other mechanisms also contribute. Mechanisms induced by H⁺ retention that might also mediate GFR decline in Nx include oxidative stress [22] and complement activation [1, 23]. The data collectively support further exploration of dietary HCO₃ as a kidney protection intervention that might be better tolerated, less expensive and possibly more effective than pharmacologic antagonists.

Contrary to our previous studies in which GFR of 2/3Nx given dietary alkali Ca(HCO₃)₂ was not different between Week 24 and Week 1 [11], GFR of 2/3Nx in the present study given dietary alkali NaHCO₃ to lower H⁺ retention to Sham levels had GFR that was slightly but significantly lower at Week 24 than that at Week 1. We used Ca(HCO₃)₂ as the alkali salt in our previous studies of 5/6Nx, because tail-cuff blood pressure was much higher in 5/6Nx given NaHCO₃ than Ca(HCO₃)₂ (155 ± 8 versus 117 ± 6, n = 8, P < 0.01) [11]. We chose NaHCO₃ as the alkali salt because it is the more likely salt to be used for dietary acid reduction [24] and shows GFR preservation in patients with reduced GFR but no metabolic acidosis [9]. In the present study, tail-cuff blood pressure was higher in 2/3 Nx given NaHCO₃ compared with that in not given NaHCO₃ (Tables 2 and 3) and was lower in 2/3 Nx given valsartan with NaHCO₃. Because blood pressure was higher in 2/3Nx of the present study given NaHCO₃ than that in 2/3Nx previously given Ca(HCO₃)₂, we hypothesize that higher blood pressure in the present study limited GFR conservation as has been described in CKD patients [25].

The mechanism of H⁺ retention in the setting of reduced GFR is not clear. This phenomenon appears to be systemic in nature, not just in the kidney cortex as reported presently, but is also present in skeletal muscle of 2/3Nx animals by microdialysis [11] and appears present in patients with reduced GFR and no metabolic acidosis detected with indirect techniques [26]. Although patients with chronically reduced GFR, like Nx, can mount the same U_{NAE} [8] and without [9] metabolic acidosis. Thus, alkali salts might synergistically reduce kidney AII effect when combined with anti-AII drugs.

| Table 4. Effect of valsartan discontinuation on GFR |
|-----------------|-----------------|-----------------|
| Week 1 | Week 23 | Week 24 |
| Group 1 (µL/min) | (+) valsartan | (+) valsartan | (+) valsartan |
| 2543 ± 134 | 2135 ± 121* | 2142 ± 68* |
| Group 2 (µL/min) | (+) valsartan | (+) valsartan | (-) valsartan |
| 2554 ± 103 | 2140 ± 128* | 2170 ± 40* |

*P < 0.05, computed versus Week 1, ANOVA. 
**P < 0.05, computed versus Week 23, paired t.
In summary, the present study used 2/3 Nx to model patients with reduced GFR and progressive GFR decline despite no metabolic acidosis to show that H+ retention mediated GFR decline through AII receptors. Dietary NaHCO3 but not AII receptor antagonism reduced H+ retention and NaHCO3 conserved GFR better than AII receptor antagonism and better than combined AII, endothelin A and aldosterone receptor antagonism. These studies support continued testing the comparatively inexpensive, safe and well-tolerated intervention of dietary HCO3 as an adjunct to blood pressure reduction and anti-AII pharmacologic therapy to preserve GFR in patients with reduced GFR at presentation.

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CONFLICT OF INTEREST STATEMENT

The results of this paper have not been published previously in whole or in part, except in abstract form.

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Impaired expression of key molecules of ammoniagenesis underlies renal acidosis in a rat model of chronic kidney disease

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ABSTRACT

Background. Advanced chronic kidney disease (CKD) is associated with the development of renal metabolic acidosis. Metabolic acidosis per se may represent a trigger for progression of CKD. Renal acidosis of CKD is characterized by low urinary ammonium excretion with preserved urinary acidification indicating a defect in renal ammoniagenesis, ammonia excretion or both. The underlying molecular mechanisms, however, have not been addressed to date.

Methods. We examined the Han:SPRD rat model and used a combination of metabolic studies, mRNA and protein analysis of renal molecules involved in acid–base handling.

Results. We demonstrate that rats with reduced kidney function as evident from lower creatinine clearance, lower haematocrit, higher plasma blood urea nitrogen, creatinine, phosphate and potassium had metabolic acidosis that could be aggravated by HCl acid loading. Urinary ammonium excretion was highly reduced whereas urinary pH was more acidic in CKD compared with control animals. The abundance of key enzymes and transporters of proximal tubular ammoniagenesis (phosphate-dependent glutaminase, PEPCK and SNAT3) and bicarbonate transport (NBCe1) was reduced in CKD compared with control animals. In the collecting duct, normal expression of the B1 H+-ATPase subunit is in agreement with low urinary pH. In contrast, the RhCG ammonia transporter, critical for the final secretion of ammonia into urine was strongly down-regulated in CKD animals.

Conclusion. In the Han:SPRD rat model for CKD, key molecules required for renal ammoniagenesis and ammonia excretion are highly down-regulated providing a possible molecular explanation for the development and maintenance of renal acidosis in CKD patients.

Keywords: acidosis, ammoniagenesis, CKD

INTRODUCTION

Metabolic acidosis is common in chronic kidney disease (CKD) and is associated with several complications such as muscle wasting, impaired growth in children, bone disease, hypoalbuminaemia, inflammation and insulin resistance. Moreover, it has been associated with increased mortality in dialysis and non-dialysis-dependent CKD patients [1–5]. Numerous recent studies particularly highlighted the role of metabolic acidosis in the progression of CKD and the increased risk to develop end-stage renal disease [6–9]. The mechanisms of metabolic acidosis in CKD were first functionally investigated >50 years ago and it was shown that patients with metabolic acidosis and CKD demonstrate reduced excretion of ammonium, the most important...