Role of 11β-hydroxysteroid dehydrogenase 2 renal activity in potassium homeostasis in rats with chronic renal failure

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Abstract

Aldosterone concentrations vary in advanced chronic renal failure (CRF). The isozyme 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2), which confers aldosterone specificity for mineralocorticoid receptors in distal tubules and collecting ducts, has been reported to be decreased or normal in patients with renal diseases. Our objective was to determine the role of aldosterone and 11β-HSD2 renal microsome activity, normalized for glomerular filtration rate (GFR), in maintaining K^+ homeostasis in 5/6 nephrectomized rats. Male Wistar rats weighing 180-220 g at the beginning of the study were used. Rats with experimental CRF obtained by 5/6 nephrectomy (N = 9) and sham rats (N = 10) were maintained for 4 months. Systolic blood pressure and plasma creatinine (Pcr) concentration were measured at the end of the experiment. Sodium and potassium excretion and GFR were evaluated before and after spironolactone administration (10 mg·kg^{-1}·day^{-1} for 7 days) and 11β-HSD2 activity on renal microsomes was determined. Systolic blood pressure (means ± SEM; Sham = 105 ± 8 and CRF = 149 ± 10 mmHg) and Pcr (Sham = 0.42 ± 0.03 and CRF = 2.53 ± 0.26 mg/dL) were higher (P < 0.05) while GFR (Sham = 1.46 ± 0.26 and CRF = 0.61 ± 0.06 mL/min) was lower (P < 0.05) in CRF, and plasma aldosterone (Pald) was the same in the two groups. Urinary sodium and potassium excretion was similar in the two groups under basal conditions but, after spironolactone treatment, only potassium excretion was decreased in CRF rats (sham = 0.95 ± 0.090 (before) *vs* 0.89 ± 0.09 µEq/min (after) and CRF = 1.05 ± 0.05 (before) *vs* 0.37 ± 0.07 µEq/min (after); P < 0.05). 11β-HSD2 activity on renal microsomes was lower in CRF rats (sham = 0.807 ± 0.09 and CRF = 0.217 ± 0.07 nmol·min^{-1}·mg protein^{-1}; P < 0.05), although when normalized for mL GFR it was similar in both groups. We conclude that K^+ homeostasis is maintained during CRF development despite normal Pald levels. This adaptation may be mediated by renal 11β-HSD2 activity, which, when normalized for GFR, became similar to that of control rats, suggesting that mineralocorticoid receptors maintain their aldosterone selectivity.

Key words: Aldosterone; 5/6 Nephrectomy; 11β-HSD2; Potassium excretion

Introduction

Potassium homeostasis is maintained up to the advanced stage of chronic renal failure (CRF) by increased fractional potassium excretion, while the glomerular filtration rate (GFR) is decreased (1-4). Although aldosterone is the key factor involved in K^+ homeostasis, its plasma concentration in CRF varies. Both increased and normal values (5-8) have been reported. The specific renal effect of aldosterone depends on the cytoplasmic access of aldosterone to its specific mineralocorticoid receptors (9-12). These receptors bind both mineralo- and glucocorticoids with high affinity (13-16). The isozyme 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2) converts cortisol to cortisone (13-16), consequently preventing mineralocorticoid receptor stimulation by cortisol and conferring specificity for aldosterone to this receptor. The isozyme is predominantly found in target aldosterone tissues: distal convoluted tubules, cortical collecting ducts (17) and colon (18). Impaired renal 11β-HSD2 activity has been described in patients with hypoxia (19) or impaired renal function (20) and in nephrotic rats (21), leading to sodium retention, hypokalemia and salt-dependent hypertension. However, an important consideration has not been discussed in these studies. Since GFR, an index

of nephron mass, is markedly reduced in CRF, this should be taken into account when renal 11β-HSD2 activity is evaluated.

The aim of the present investigation was to study the role of aldosterone and 11β-HSD2 renal microsome activity in maintaining K^+ homeostasis in 5/6 nephrectomized rats. To address this question we evaluated renal function, systolic blood pressure (SBP), plasma aldosterone, and 11β-HSD2 renal microsome activity in 5/6 nephrectomized and sham rats.

Material and Methods

Animals

Animals were used in compliance with the animal Research Guidelines of the American Heart Association (www.americanheart.org).

Male Wistar rats weighing 180 to 220 g were used. The animals had free access to Purina Rodent Laboratory chow and water throughout the experiment. This standard diet contains 282 mmol/kg K^+, and 174 mmol/kg Na^+. Experimental renal insufficiency was induced in rats (N = 9) according to the technique of Morrison (22). The two poles of the left kidney were removed and the right kidney was excised one week later. Sham rats (N = 10) submitted to laparotomy and manipulation of the renal pedicles were used as controls.

Experimental protocol

Rats were housed in a humidity- and temperature-controlled environment with an automatic light/dark cycle of 12:12 h and studied for 4 months starting at the time of right kidney nephrectomy.

Systolic blood pressure and plasma creatinine concentration were measured every month. At the end of this period, rats were adapted to metabolic cages during a period of 4 days. Subsequently, a blood sample and 24-h urine were obtained. Urinary flow, GFR, obtained by creatinine clearance, and sodium and potassium excretion were determined. Spironolactone, an aldosterone antagonist analogue extensively used in experimental studies on rats (23-25), was administered by gavage (10 mg/kg body weight) daily for 7 days. Next, rats were returned to metabolic cages and the same experimental procedure was performed. The experimental group contained 9 rats and the sham-operated group 10 rats.

Measurement of 11β-HSD2 activity in renal microsomes

Rats were anesthetized with ether and kidneys were perfused with 0.9% NaCl, excised, decapsulated, and sliced. The slices were resuspended in 0.1 M sodium phosphate buffer containing 1.5 mM $MgCl_2$, pH 7.4 (MG buffer; 25 mL MG buffer/g tissue) and homogenized with a Potter Teflon homogenizer. Homogenates were centrifuged at 12,000 g

for 30 min. The supernatant was then centrifuged at 105,000 g for 60 min and the microsome fraction thus obtained was resuspended in MG buffer. Total protein was determined by the method of Bradford (26).

Isozyme activity was determined by measuring the conversion rate of ^{3}H-corticosterone to ^{3}H-11-dehydrocorticosterone (27). Microsomal suspensions containing 250 µg protein/mL were incubated in 250 µL MG buffer containing 14 nM ^{3}H-corticosterone and 400 µM NAD^+ for 10 min at 37°C and the reaction was stopped by the addition of ethyl acetate. Steroids were extracted, and then separated by thin layer chromatography using chloroform-ethanol (92:8). The ^{3}H-corticosterone and ^{3}H-11β-dehydrocorticosterone were eluted and radioactivity was counted. Enzyme specific isozyme activity is reported as nmol·min^{-1}·mg protein^{-1}.

Analytical methods

Aldosterone was measured in blood samples by radioimmunoassay, SBP was determined by tail plethysmography in awake rats (28), Na^+ and K^+ concentrations were determined in urine samples by flame photometry, and plasma and urinary creatinine were determined by a modified Jaffe method, which prevents nonspecific reaction (29).

Statistical analysis

Data are reported as means ± SEM. To test for statistically significant differences (P < 0.05), the Student t-test for paired or unpaired data, as appropriate, was performed.

Results

Rats with 5/6 nephrectomy developed a significant deterioration of renal function, as shown in Table 1. In fact, after 4 months at the end of the experiments, plasma creatinine concentration and the SBP were higher (P < 0.05) and GFR was significantly lower in CRF rats. The validity of the use of creatinine clearance to evaluate GFR in Wistar rats was demonstrated (30). Although CRF effectively developed

Table 1. Plasma creatinine, systolic blood pressure, glomerular filtration rate, and plasma aldosterone levels of sham and chronic renal failure rats.

	Sham (N = 10)	CRF (N = 9)
Pcr (mg/dL)	0.42 ± 0.03	2.53 ± 0.26*
SBP (mmHg)	105 ± 8	149 ± 10*
GFR (mL/min)	1.46 ± 0.26	0.61 ± 0.06*
Pald (pg/mL)	1,746 ± 191	1,615 ± 113

Data are reported as means ± SEM. Measurements were made 4 months after 5/6 nephrectomy. CRF = chronic renal failure; Pcr = plasma creatinine; SBP = systolic blood pressure; GFR = glomerular filtration rate; Pald = plasma aldosterone. *P < 0.05 *vs* sham (Student t-test for unpaired data).

after 5/6 nephrectomy, plasma K^+ concentration (Table 2) continued to be normal.

Although urinary sodium and potassium excretion did not differ in either group under basal conditions (Figure 1), fractional Na^+ excretion [sham: $0.19 \pm 0.02\%$ (N = 10), CRF: $0.45 \pm 0.09\%$ (N = 9)], and fractional K^+ excretion [sham: $13.25 \pm 1.26\%$ (N = 10), CRF: $37.25 \pm 3.50\%$ (N = 9)] were significantly higher (P < 0.01) in 5/6 nephrectomized rats. Thus, a tubular adaptation allowing the remaining nephrons to preserve the electrolyte balance must have been developed in the nephrectomized group. In contrast, no difference was found in plasma aldosterone between sham and CRF rats (Table 1), although, after treatment with spironolactone, the two groups responded in different ways (Figure 1). Sodium excretion was not modified in either group and K^+ excretion did not change in sham rats but was substantially decreased in CRF rats (P < 0.05). Thus, plasma potassium concentration increased significantly only in CRF rats treated with spironolactone, as shown in Table 2. This specific effect of spironolactone suggests an adaptive response of mineralocorticoid receptors in the distal nephrons of CRF rats. When 11β-HSD2 renal microsome activity was measured, a lower value was obtained for the CRF group (P < 0.05; Figure 2). However, when 11β-HSD2 activity was normalized for mL GFR, an index of remnant nephron mass, there was no difference between groups.
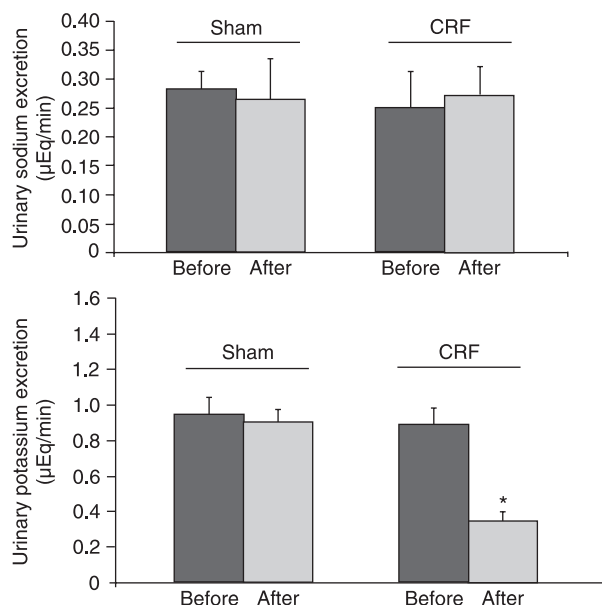
Discussion

In the present study, plasma Na^+ and K^+ concentrations were similar in sham and CRF rats, showing that the electrolyte balance was preserved throughout the 4-month study. The compensation of renal function in order to maintain electrolyte homeostasis while renal disease is developing has been well documented (31,32). The "intact nephron hypothesis" (31) considers a special mechanism in remnant nephrons that elevates GFR and enlarges the tubule. This specific adaptation facilitates Na^+, K^+ and water handling (33,34). On this basis, we demonstrated that fractional Na^+ and K^+ excretion was significantly higher in 5/6 nephrectomized rats. We emphasize the low relationship of Na^+ and K^+ excretion in sham rats, of the order of 1 to 4, when the expected one was 2/1 (35). It should be pointed out that our rats were fed a standard commercial Purina Rodent Laboratory diet, which in comparison with the occidental human diet (36) contains high K^+ and low Na^+ (see Methods).

Plasma aldosterone levels were the same in the two

Table 2. Effect of spironolactone on plasma potassium of sham and chronic renal failure rats.

	Plasma potassium (mEq/L)	
	Before spironolactone	After spironolactone
Sham (N = 10)	4.77 ± 0.26	5.32 ± 0.25
CRF (N = 9)	4.89 ± 0.43	5.91 ± 0.31*

Data are reported as means ± SEM. Measurements were made 4 months after 5/6 nephrectomy or sham operation. CRF = chronic renal failure. Spironolactone was administered by gavage (10 mg·kg^{-1}·day^{-1} for 1 week). *P < 0.05 *vs* before (Student *t*-test for paired data).

Figure 1. Urinary sodium and potassium excretion of sham and chronic renal failure (CRF) rats before and after spironolactone treatment (10 mg·kg^{-1}·day^{-1} for 1 week). Measurements were made 4 months after 5/6 nephrectomy or sham operation. Data are reported as means ± SEM. *P < 0.05 *vs* before treatment (Student *t*-test for paired data).
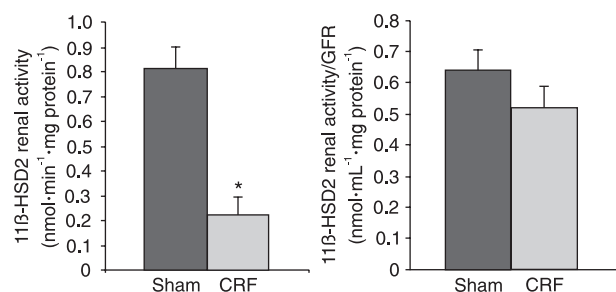
Figure 2. Renal microsome 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2) activity and 11β-HSD2 activity normalized for mL glomerular filtration rate (GFR) of sham and chronic renal failure (CRF) rats. Measurements were made 4 months after 5/6 nephrectomy or sham operation. Data are reported as means ± SEM. *P < 0.05 *vs* sham (Student *t*-test for unpaired data).

groups but spironolactone treatment only modified K$^+$ excretion in 5/6 nephrectomized rats (Figure 1). We may speculate that during chronic renal failure, mineralocorticoid receptors develop a higher aldosterone sensitivity. This could be an important adaptive mechanism, which allows remnant tubular nephrons to handle the K$^+$ overload.

As pointed out above, the apparent difference in the effect of spironolactone on potassium and sodium excretion can be explained by the effect of the low Na$^+$ and high K$^+$ content of the Purina rat diet when compared to the occidental human diet, which have more sodium than potassium (36). Accordingly, Malnic et al. (37) found that, in spite of the low sodium content of the diet, the distal nephron handling of potassium might not be affected.

The 11β-HSD2 renal microsome activity was significantly lower in our CRF animals. Impaired activity of this isozyme has been demonstrated in hypoxia (19) and in renal disease (20,21), syndromes that develop with abnormal handling of potassium and sodium and high blood pressure. These observations led us to hypothesize that the decreased 11β-HSD2 may improve the access of glucocorticoids to mineralocorticoid receptors, leading to hypertension and Na$^+$ retention. However, contradictory results have been reported by others. Bistrup et al. (21), working with a puromycin aminonucleoside model of nephrotic syndrome, have shown that, although renal 11β-HSD2 was down-regulated, total protein expression was unchanged. Furthermore, working with aging WAG/rij rats, a strain without increased blood pressure or that does not develop kidney disease with age,

Audigè et al. (38) did not obtain any evidence suggesting enhanced kidney gene expression or activity of 11β-HSD2. They concluded that endogenous 11β-HSD2 is able to cope with the increased corticosterone concentrations characteristic of the aging process. Yet, the progressive loss of nephron mass associated with advanced age (39,40), which must impair GFR, was not taken into account by Audigè et al. (38). In the present investigation, GFR was markedly reduced as a consequence of a 5/6 reduction in the renal mass (Table 1). When we normalized 11β-HSD2 activity for mL GFR, we did not observe a difference between sham and CRF rats (Figure 2). Thus, when reduction of nephron mass is considered, 11β-HSD2 activity appears to be sufficient to maintain mineralocorticoid receptor specificity in the remnant nephrons.

In conclusion, the present study demonstrates one of the mechanisms that may contribute to K$^+$ homeostasis up to the advanced stage of renal failure. Despite normal aldosterone plasma level, plasma K$^+$ concentration remained normal. The isozyme 11β-HSD2 can, when taking the reduced nephron mass into account, be able to preserve K$^+$ balance by protecting mineralocorticoid receptors.

Acknowledgments

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