

Effect of Chronic Experimental Renal Insufficiency on Urate Metabolism¹

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ABSTRACT

The rise in plasma uric acid (UA) in chronic renal failure (CRF) is quite limited. This may be due to either increased extrarenal excretion, diminished biosynthesis, and/or enhanced degradation of uric acid. The intestinal flux studies revealed a striking modification of urate transport from no net flux to a net secretory flux in the jejunum and from a basal net absorptive to a net secretory flux in the colon of CRF animals. In addition, CRF animals showed a marked reduction in hepatic, renal, and enteric tissue xanthine oxidase activity and no significant change in tissue uricase activity. The correction of anemia with erythropoietin did not significantly alter the plasma concentration or urinary excretion of urate. Thus, enhanced enteric excretion and depressed production of uric acid (reduced xanthine oxidase activity) may account for the lack of significant hyperuricemia in CRF.

Key Words: Uric acid, intestinal transport, chronic renal failure, uricase, xanthine oxidase

Despite a marked reduction in its urinary clearance, the rise in plasma uric acid concentration in chronic renal failure (CRF) is quite limited (1,2). The possible reasons for this phenomenon may include a rise in fractional uric acid excretion by the remaining nephrons, increased extrarenal elimination, enhanced degradation, and/or decreased production of uric acid. In a recent study, we demonstrated altered enteric transport of uric acid in rats with chronic renal insufficiency (3). This study confirmed these earlier observations and further explored the effects of experimental CRF on uric acid metabolism in rats. Renal handling, intestinal transport, tissue xanthine oxidase, and uricase activities were compared in 5/6 nephrectomized (CRF) and normal control rats. In addition, the possible role of the associated hypoproliferative anemia on uric acid metabolism was studied

by including a group of erythropoietin (EPO)-treated CRF animals.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 275 to 300 g were used. Animals were randomized into CRF and normal control groups. Animals assigned to the CRF group were subjected to a right nephrectomy followed by a two-thirds left nephrectomy 4 days later as described previously (3). Animals were fed regular rat chow (Purina Mills, Brentwood, MO), and daily food intake and hematocrit were monitored. In an attempt to discern the possible effect of EPO-deficiency anemia on urate production, two additional groups of CRF animals were studied. One group received intraperitoneal injections of recombinant EPO (150 U/kg twice weekly) to prevent the associated anemia (CRF-EPO group), whereas the second group received placebo injections.

Six weeks after surgery, the animals were subjected to separate series of experiments intended to address the effect of CRF on distinct aspects of urate metabolism including renal handling, intestinal transport, and the key enzymes involved in urate synthesis (*i.e.*, xanthine oxidase) and degradation (*i.e.*, uricase). Blood and urine were collected from animals in each group for the determination of hematocrit, uric acid (Sigma Kit #685), and creatinine (Sigma Kit #555A; Sigma Chemical Co., St. Louis, MO).

At the end of the 6-wk observation period, with the rats under general anesthesia, liver, kidneys, and intestinal tissues were removed for the quantitation of enzyme activities and intestinal flux studies. Flux measurements were conducted on isolated, short-circuited jejunum, ileum, and colon as described in detail previously (3). Tissue conductance (G_T , millisiemens per square centimeter) was calculated as the ratio of the open-circuit potential (millivolts) to short-circuit current I_{SC} (microamperes per square centimeter). Unidirectional fluxes of urate were determined by the addition of [¹⁴C]urate (Dupont, NEN Research Products, Boston, MA) in trace amounts to one bathing solution and the measurement of its appearance in the opposite saline. Tracer activity (disintegrations per minute) was measured by standard liquid scintillation spectrometry (Beckman LS 9000, Beckman Instruments, Inc. Fullerton, CA). Net fluxes were taken as the difference between the two unidirectional fluxes in conductance-matched tissue pairs (G_T not differing by more than $\pm 20\%$). Fluxes and electrical parameters were measured at 20-min intervals for a 60-min period.

Approximately 600 mg of liver and kidney and 500 to 800 mg of intestinal mucosal scrapings were each homogenized (30-s pulse) in 10 mL of a cold buffer solution with a Brinkmann Polytron tissue/cell disrupter (Model PT 10/35, Brinkmann Instruments Inc., Westbury, NY). The buffer solution contained 300 mM mannitol, 20 mM *N*-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), and 13.3 mM Tris (pH = 7.4). Before storage at -80°C , aliquots of these homogenates were appropriately diluted for both enzyme activity measurements and protein determination by use of the Bradford method (Bio-Rad Protein Kit; Bio-Rad, Richmond, CA).

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Xanthine oxidase activity was determined (4) in aliquots of tissue homogenates by measuring an increase in absorbance at 292 nm due to urate formed from excess xanthine used as a substrate in a pyrophosphate buffer (0.1 M, pH 8). A unit of xanthine oxidase is defined as that amount forming 1 nmol of urate per minute per milligram of protein at 21°C. The subsequent conversion of urate to allantoin and the influence of this reaction on xanthine oxidase activity measurement are presumed negligible and have been ignored because in the presence of excess xanthine, the maximum initial velocity of the reaction is being measured.

Uricase activity was similarly determined by measuring the decrease in absorbance at 292 nm in a glycine buffer (0.5 M, pH 9.4) reaction mixture containing excess urate as a substrate with a Beckman DU-64 spectrophotometer. A unit of uricase is defined as that amount that oxidizes 1 nmol of urate to allantoin per minute per milligram of protein at 21°C.

Statistical Methods

Results are presented as the mean ± SE for the number (N) of animals or tissues or tissue pairs in the transport studies. The data obtained from the measurements of plasma and urine specimens were analyzed by the use of a t test to establish differences between means (Tables 1 and 2). Statistical analysis of the transport studies (Figure 1) was performed by a one-way analysis of variance in conjunction with Duncan's multiple range test. The data obtained from the enzyme studies (Tables 3 and 4) were analyzed by a two-way analysis of variance in conjunction with Duncan's multiple range test. Differences were considered significant if P ≤ 0.05.

RESULTS

Renal Urate Handling

Compared with the baseline values, the CRF animals showed no significant change in plasma urate concentration 6 wk after subtotal nephrectomy despite marked reductions in urate clearance and urinary urate excretion (Table 1). In contrast, the fall in creatinine clearance was associated with an expected rise in plasma creatinine concentration and a normal rate of urinary creatinine excretion in the CRF group (Table 1). Accordingly, a significant fall in the fractional excretion of uric acid in the CRF animals (14.5 ± 5.5 [baseline] versus 5.9 ± 1.0% [6 wk] in the CRF

TABLE 2. Hematocrit values in CRF rats receiving placebo or EPO (150 U/kg twice weekly) at baseline and 6 wk postnephrectomy

Group	Baseline	6 Wk
CRF (N = 12)	46.41 ± 0.86	41.50 ± 0.88 ^a
CRF-EPO (N = 12)	47.41 ± 0.61	47.25 ± 2.59

^a Significantly different from baseline value, P < 0.05.

group and 13.0 ± 1.9 [baseline] versus 4.8 ± 0.6% [6 wk] in the EPO-CRF group) was observed. Daily food intake and gain in body weight over the 6 wk after partial nephrectomy were similar in CRF rats compared with normal rats.

Effect of EPO Administration

As expected, the CRF group showed a significant reduction in hematocrit, and the biweekly administration of EPO effectively restored hematocrit to the pre-nephrectomy values (Table 2). A comparison of data obtained in the placebo- and EPO-treated groups showed no significant difference in either plasma concentration, urinary excretion rate, or fractional excretion of urate (Table 1).

Enteric Urate Transport

Significant alterations in urate fluxes were previously reported (3) and confirmed here in intestinal tissues removed from the CRF rats. Small changes in the unidirectional fluxes of urate led to a significant net secretion across the jejunum (Figure 1). This was not accompanied by any changes in the accompanying electrical parameters. The ileal transport of urate was not altered in CRF (Figure 1). In the colonic segment, a significant increase in the unidirectional serosal to mucosal flux of urate resulted in reversing basal net absorption to net secretion (Figure 1). The 2.5-fold increase in I_{sc} across these tissues is consistent with the results of an earlier study demonstrating that electrogenic chloride secretion was also induced (3).

TABLE 1. Plasma concentrations, urinary excretion rates, and renal clearances of creatinine and urate in the CRF group and EPO-treated CRF (CRF-EPO) animals at baseline and 6 wk postnephrectomy

Group	Plasma (mM)		Excretion (μM/24 h)		Clearance (mL/min)	
	Baseline	6 Wk	Baseline	6 Wk	Baseline	6 Wk
Creatinine						
CRF (N = 12)	0.04 ± 0.003	0.10 ± 0.004 ^a	90.8 ± 4.97	128.2 ± 8.4	1.58 ± 0.19	0.93 ± 0.07 ^a
CRF-EPO (N = 12)	0.04 ± 0.004	0.11 ± 0.006 ^a	84.7 ± 5.0	118.5 ± 7.5	1.59 ± 0.17	0.74 ± 0.054 ^a
Urate						
CRF (N = 14)	0.047 ± 0.007	0.062 ± 0.010	8.94 ± 1.66	4.51 ± 0.62 ^a	0.23 ± 0.05	0.05 ± 0.010 ^a
CRF-EPO (N = 14)	0.045 ± 0.006	0.063 ± 0.023	8.87 ± 0.97	4.17 ± 0.44 ^a	0.22 ± 0.03	0.03 ± 0.004 ^a

^a P < 0.05 versus baseline values.

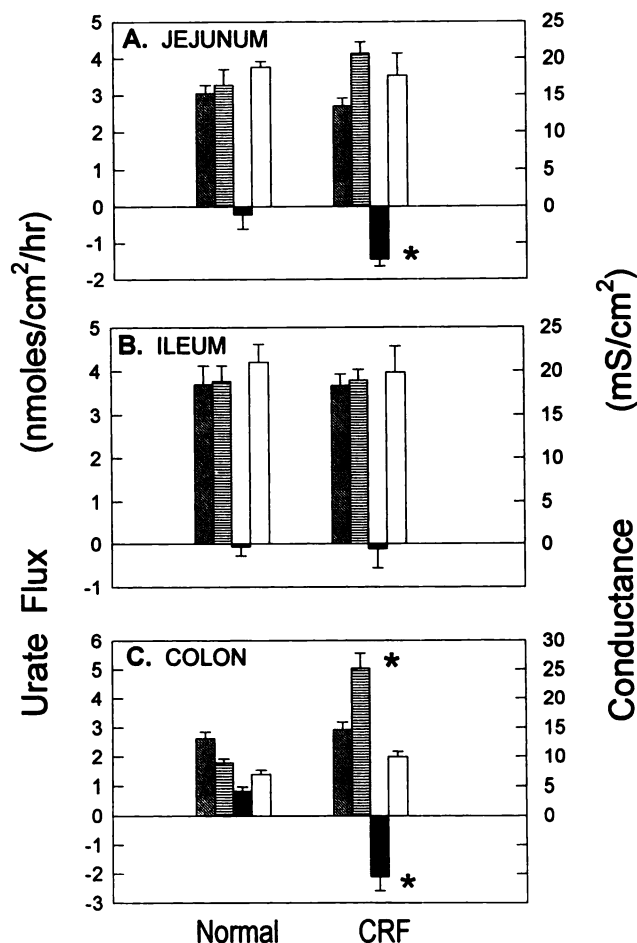


Figure 1. (a to c) Urate fluxes across jejunum, ileum, and colon of normal and CRF rats. Data include: serosa to mucosa (▨), mucosa to serosa (■), and net flux of urate (□), respectively. G_t (□) is tissue conductance. An asterisk indicates a significant difference from normal ($P \leq 0.05$). Results obtained from a minimum of six tissue pairs from at least four animals in each series.

Tissue Xanthine Oxidase Activity

The results of measurements of xanthine oxidase activity in various tissue homogenates are presented in Table 3. Basal xanthine oxidase activity in the liver homogenates from normal rats was at least three times higher than that found in the kidney and intestinal tissue homogenates from the same animals. Both CRF groups showed a marked reduction in xanthine oxidase activity in all tissues studied, *i.e.*, the liver, kidney, jejunum, ileum, and colon tissues, as compared with the normal control group (Table 3). The reduction in xanthine oxidase activity was greatest in hepatic tissue ($\downarrow 70\%$) and least in renal tissue ($\downarrow 40\%$). Comparable reductions ($\downarrow 60\%$) in xanthine oxidase activity were apparent in the homogenates of the three intestinal segments. No significant difference was found in tissue xanthine oxidase activities between EPO- and placebo-treated animals with CRF.

TABLE 3. Xanthine oxidase^a activity in homogenates of liver, kidney, jejunum, ileum, and colon removed from normal, CRF, and EPO-treated CRF rats

Tissue	Normal	CRF	CRF-EPO
Liver	7.50 ± 0.60 (7)	2.43 ± 0.84 ^b (8)	2.97 ± 0.78 ^b (7)
Kidney	1.92 ± 0.27 (10)	1.19 ± 0.19 ^b (8)	1.25 ± 0.20 ^b (8)
Jejunum	2.38 ± 0.30 (8)	0.99 ± 0.06 ^b (8)	1.16 ± 0.23 ^b (8)
Ileum	1.99 ± 0.83 (13)	0.76 ± 0.08 ^b (8)	0.85 ± 0.18 ^b (8)
Colon	2.49 ± 0.23 (14)	1.08 ± 0.17 ^b (8)	1.54 ± 0.33 ^b (8)

^a A unit of xanthine oxidase activity is that amount forming 1 nmol of uric acid per minute per milligram of protein at 21°C.

^b Significantly different from normals ($P \leq 0.05$) for duplicate assays from (N) rats.

TABLE 4. Uricase^a activity in homogenates of liver, kidney, jejunum, ileum, and colon removed from normal, CRF, and EPO-treated CRF rats

Tissue	Normal	CRF	CRF-EPO
Liver	6.37 ± 0.62 (10)	7.44 ± 0.47 (11)	6.09 ± 0.86 (8)
Kidney	1.59 ± 0.28 (10)	1.93 ± 0.16 (11)	2.24 ± 0.42 (7)
Jejunum	2.43 ± 0.48 (9)	1.84 ± 0.13 (11)	1.89 ± 0.23 (8)
Ileum	1.37 ± 0.22 (15)	1.99 ± 0.22 (11)	1.75 ± 0.16 (8)
Colon	2.04 ± 0.32 (14)	2.87 ± 0.30 (11)	3.71 ± 0.53 ^b (8)

^a A unit of uricase activity is that amount that oxidizes 1 nmol of uric acid to allantoin per minute per milligram of protein at 21°C.

^b Significantly different from normals ($P \leq 0.05$) for duplicate assays from (N) rats; not different from CRF group.

Tissue Uricase Activity

Uricase activity in the liver was markedly higher than that of the other tissues examined (Table 4). There were no significant alterations in the uricase activity of any of the tissues removed from CRF animals when compared with those obtained from the normal controls. EPO therapy had no discernible effect on hepatic, renal, jejunal, or ileal tissue uricase activities. Interestingly, mean colonic uricase activity was significantly elevated in the EPO-treated CRF group and insignificantly increased in the placebo-treated CRF animals as compared with that found in the control group.

Effect of Uremic Environment

No significant difference was found in the enzymatic activity of a commercial xanthine oxidase preparation (Sigma Chemical Co.) measured in the presence of 20 μ L of uremic plasma when compared with that ob-

served in the presence of normal plasma (Figure 2). These findings tend to exclude the presence of an inhibitor of xanthine oxidase in the uremic plasma.

DISCUSSION

Several mechanisms, separately or in combination, can potentially account for the absence of significant hyperuricemia in spite of the marked reduction in urinary uric acid excretion observed in the CRF animals (Table 1). These include: (1) enhanced extrarenal excretion, (2) diminished biosynthesis, and/or (3) increased degradation of uric acid. In this study, we confirmed the observations made in an earlier study demonstrating the role of enteric transport as an extrarenal route of urate excretion in experimental CRF (3). The secreted urate is assumed to be readily degraded by an increased population of colonic microorganisms (5).

In addition, when we further explored aspects of urate metabolism, we found a marked reduction of hepatic, renal, jejunal, ileal, and colonic xanthine oxidase activity in the CRF animals. Inherited deficiency (*i.e.*, hereditary xanthinuria) and pharmacologic inhibition of this enzyme (*e.g.*, allopurinol therapy) are associated with a marked reduction of endogenous production and, consequently, diminished plasma concentration and urinary excretion of urate (6,7). Thus, the observed depression of tissue xanthine oxidase activity may be indicative of the decreased endogenous production of urate. If true, the latter may, in part, contribute to the lack of significant hyperuricemia in the face of diminished urinary urate excretion in the CRF animals. The available data, however, do not allow a definitive conclusion as to the

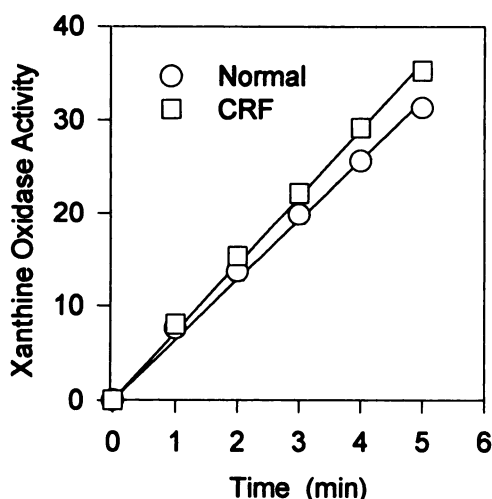


Figure 2. Xanthine oxidase activity in the presence of 25 μ L of normal and CRF plasma. Values are means \pm SE for triplicate determinations of four pooled plasma samples. Each pooled sample contained equal volumes of plasma from three rats in each condition. Error bars are contained within the symbols.

mechanism of the CRF-induced depression of xanthine oxidase activity. It is not clear if the reduction in enzyme activity is due to the reduced total quantity or the impaired functional capacity of the enzyme. However, our *in vitro* incubation of purified xanthine oxidase with the plasma obtained from the study groups revealed no inhibitory effect of uremic plasma. Thus, the observed reduction of tissue xanthine oxidase activity in the CRF animal is unlikely due to the inhibitory effect of compound(s) present in the uremic plasma.

Although no significant difference was found in the uricase activities of either hepatic, renal, jejunal, or ileal tissues between the study groups, the mean uricase activity of the colonic tissue was significantly increased in the EPO-treated CRF group and insignificantly elevated in the placebo-treated CRF animals when compared with the normal group. The resident population of substrate-specific (urate) microorganisms is known to be elevated in CRF (8,9). It is, therefore, conceivable that uricase liberated from this source during the tissue homogenization is responsible for the observed increase in the enzyme activity. Thus, our experiments appear to preclude a pervasive CRF-induced change in the tissue biodegradation of urate in rats. Unlike rats, humans do not possess uricase, and as such, consideration of the effect of uremia on tissue uricase is of no relevance to humans.

CRF animals exhibited a significant reduction in hematocrit consistent with the EPO-deficiency anemia of CRF (10). This prompted the question of whether this diminished erythropoiesis significantly affected purine turnover and hence the endogenous production, urinary excretion, or plasma concentration of uric acid. Comparison of the results obtained in the EPO- and placebo-treated CRF groups revealed no significant differences in either plasma urate concentration, urinary urate excretion, tissue xanthine oxidase, or uricase activities. These observations suggest that the mild anemia observed in CRF animals did not play a major role in the observed alterations of urate production and metabolism.

It is conceivable that diminished food intake associated with CRF-induced anorexia may, in part, contribute to reduced urate production from the dietary sources of purine. However, the average daily food intake in the CRF group was comparable with that of the normal control group.

Thus, the reduction in urinary urate clearance in CRF rats appears to be offset by the enhanced enteric excretion of urate along with depressed tissue urate production. These changes may account for the absence of severe hyperuricemia and the low incidence of gout in CRF (2).

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