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Altered renal elimination of organic anions in rats with chronic renal failure

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Abstract

The progress of chronic renal failure (CRF) is characterized by the development of glomerular and tubular lesions. However, little is known about the expression of organic anions renal transporters. The objective of this work was to study, in rats with experimental CRF (5/6 nephrectomy), the expression of the organic anion transporter 1 (OAT1) and organic anion transporter 3 (OAT3) and their contribution to the pharmacokinetics and renal excretion of *p*-aminohippurate (PAH). Two groups of animals were used: Sham and CRF. Six months after surgery, systolic blood pressure and plasma creatinine concentrations were significantly higher in CRF groups. CRF rats showed a diminution in: the filtered, secreted and excreted load of PAH; the systemic clearance of PAH; the renal OAT1 expression; and the renal Na–K-ATPase activity. No remarkable modifications were observed in the OAT3 expression from CRF kidneys. The diminution in the systemic depuration and renal excretion of PAH may be explained by the decrease in its filtered and secreted load. The lower OAT1 expression in remnant renal mass of CRF rats or/and the lower activity of Na–K-ATPase might justify, at least in part, the diminished secreted load of this organic anion.
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1. Introduction

The progression of chronic renal failure (CRF) is characterized by the development of glomerular and tubular lesions about which multiple factors can be involved [1]. Regarding renal tubules, numerous studies have been performed to understand the pathological process [2], but it is less known about changes in tubular function. In the experimental model of subtotal nephrectomy, functional tubular changes and elevated filtration rate per remaining nephron are expected because of compensatory renal hypertrophy [3]. The increase per nephron of several tubular activities is proof of it [4]. Some authors have provided evidences that tubular dysfunction occurs in CRF [5–7]. It has been demonstrated that CRF induced a reduction in the expression and/or activity of several enzymes located in brush-border membranes and of type II Na–Pi cotransporter [5]. Other studies have also reported reduced expression per cellular unit of other enzymes or transporters present in the proximal and distal parts of the nephron [6,7].

The tubular secretion of organic anions is an important function of the kidney by eliminating potentially toxic organic anions from the body [8]. A number of drugs, such as β-lactamic antibiotics, diuretics, nonsteroidal anti-inflammatory drugs, and several antiviral drugs are also classified as organic anions, therefore, the renal organic anion transport system plays a key role in the pharmacokinetics of these drugs [8]. Organic anions are taken up from the

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peritubular plasma across the basolateral membrane and effluxed into the tubular fluid across the luminal membrane. The systems involved in organic anion secretion can be functionally subdivided in the well-characterized sodium-dependent *p*-aminohippurate (PAH) system and a recently discovered sodium independent system. Both systems mediate two membrane translocation steps arranged in series: uptake from blood across the basolateral membrane of renal epithelial cells, followed by efflux into urine across the apical membrane. A lot of organic anions transporters have been recently cloned. Among them, there were the organic anion transporter 1 (OAT1) and the organic anion transporter 3 (OAT3), which are considered the principal contributors to the classical renal organic anion secretory process [8–11]. Both OAT1 and OAT3 support OA/α-KG exchange [8]. PAH is a substrate for both rodent and human orthologs of OAT1 and OAT3 [8–11]. In the apical membrane, multidrug resistance protein 2 (MRP2) has been described as one of PAH transporters [12,13]. Recently, multidrug resistance protein 4 (MRP4) has been identified as a novel PAH transporter [14]. Organic anion transporters k1 and k2 (OAT-K1 and OAT-K2) are also present in luminal membrane of proximal tubule, but they do not transport PAH [15]. The functional and molecular changes in these organic ion transporters would result in impaired renal excretion of drugs, thereby causing unexpected adverse effects of administered ionic drugs. Whether drug secretion by renal tubules is modified in CRF is questioned because of frequent accumulation of various toxins in CRF. It is generally acknowledged that some anionic drugs should be used in lower dosages or with longer inter-dose intervals to prevent adverse effects in CRF. Thus, information regarding organic anion transporters (which play a significant role in their elimination) in CRF would be useful to determine the optimal use of drugs in progressive renal failure. In these regards, Laouari et al. [16] have found that CRF resulted in enhanced MRP2 expression, and Takeuchi et al. [17] have demonstrated that OAT-K1 and OAT-K2 expressions were depressed in CRF. As OAT1 and OAT3 also play an important role in renal drug secretion, the purpose of the present study was to examine the effects of chronic renal failure on the expression of OAT1 and OAT3 in rats, and the contribution of these effects on the pharmacokinetic and renal excretion of PAH.

2. Materials and methods

2.1. Experimental protocol

Two groups of rats were used: (1) Wistar rats that underwent sham operation with manipulation of pedicle (Sham). (2) Experimental chronic renal failure (CRF) was induced in male Wistar rats of 45 days old according to the technique of Morrison et al. [18] as we have previously described [19]. The two poles of the left kidney were removed and, 1 week later, the right kidney was excised. Rats of both groups were studied for 24 weeks starting at the time of right kidney uninephrectomy or sham operation.

2.2. Assays for chronic insufficiency development

Body weight and systolic blood pressure (SBP) were monitored weekly, and the plasma creatinine (PCr) concentrations were measured every week. SBP was measured in awake rats by tail plethismography as previously described [20,21]. PCr was measured by a modified Jaffe's method [22]. Histopathology of kidneys from Sham and CRF rats was performed after fixing in 10% neutral buffered formaldehyde solution for 4 h and embedding in paraffin, then 4 μm thick sections were processed for routine staining with hematoxylin and eosin.

2.3. Preparation of kidney homogenates

Kidneys from Sham ($n=4$) and CRF ($n=4$) were rapidly removed, the renal tissue was cleaned, dried, weighed, and placed in saline. Then, the tissue was thoroughly homogenized in 250 mM sucrose, 10 mM triethanolamine, and 0.1 mg/mL phenylmethylsulfonyl fluoride (PMSF). Protein quantification of samples was performed using the Sedmak and Grossberg method [23]. These homogenates were used for measuring alkaline phosphatase abundance and Na–K-ATPase activity.

2.4. Preparation of basolateral membrane (BLM)

The preparations of BLM from Sham and CRF rats were done by a modification of the method described by Jensen and Berndt [24] as previously reported by us [25,26]. Approximately 4 g of kidney tissue was placed in a Dounce homogenizer containing 50 mL of 250 mM sucrose, 5 mM Tris–Hepes pH 7.40. After four gentle strokes with the loose fitting pestle, the suspension was homogenized further with a motor-driven Teflon pestle (600 rpm/5 strokes) and spun down for 15 min at $1200 \times g$. The supernatant was aspirated and spun for 15 min at $22{,}000 \times g$. The fluffy beige upper layer of the resulting pellet (crude plasma membranes) was resuspended in about 1 mL of supernatant and added to 19 mL of buffered sucrose. The membrane suspension was homogenized gently through a 16-gauge gavage needle followed by the addition of 3.7 mL of 100% Percoll. The Percoll-membranes mixture was spun for 30 min at $39{,}250 \times g$. The top clear layer was discarded and the topmost dark band was removed. This layer was composed primarily of basolateral membranes as established by marker enzymes analysis. BLM were brought up in KCl buffer (85 mM KCl, 83 mM sucrose, 2 mM Hepes–Tris pH 7.40) at a ratio of 8 mL/g original wet weight. Then, BLM were pelleted at $30{,}000 \times g$ for 30 min and washed three times with the KCl buffer indicated. Finally, BLM were resuspended in 300 μL of 250 mM manitol, 10 mM Hepes–

Tris, pH 7.40. Aliquots of the membranes were stored immediately at −70 °C until use (no more than 2 weeks after membrane preparations). Each preparation represented renal tissue from six animals. Protein quantification of samples was performed using the method of Sedmak and Grossberg [23].

2.5. Immunoblot technique

Samples (Homogenates or BLM) were boiled for 3 min in the presence of 1% 2-mercaptoethanol/2% SDS (sodium dodecyl sulfate). Samples were applied to a 8.5% polyacrylamide gel, separated by SDS-PAGE, and then electroblotted to nitrocellulose membranes. For comparison between groups of animals, staining with ponceau red revealed that equal quantities of proteins were deposited [26–28]. The nitrocellulose membranes were incubated overnight with 5% nonfat dry milk in phosphate-buffer saline containing 0.1% Tween 20 (PBST). After being rinsed with PBST, the membranes were then incubated with a commercial polyclonal antibody against OAT1 (1.25 μg/mL) or against alkaline phosphatase (diluted 1:2500) for 2 h or with non-commercial rabbit polyclonal antibody against rat OAT3 (at a dilution of 1:1000) overnight. Membranes were incubated for 1 h with a peroxidase coupled goat anti-rabbit IgG (Bio-Rad) after further washing with PBST. Blots were processed for detection using commercial kits (Opti-4CN, Bio-Rad for OAT1 and ECL enhanced chemiluminescence system; Amersham for alkaline phosphatase and OAT3). An absorption test was also performed. The OAT1 peptide (1.25 μg/mL) or OAT3 peptide (0.50 mg/mL) was added to the OAT1-antibody solution or OAT3-antibody solution respectively and incubated for 2 h. Using these preabsorbed antibodies, Western blot analyses were performed as described above. A densitometric quantification of Western blot signal intensity of membranes was performed.

2.6. Immunohistochemistry microscopy

The kidneys were briefly perfused with saline, followed by perfusion with periodate–lysine–paraformaldehyde solution (0.01 M $NaIO_4$, 0.075 M lysine, 0.0375 M phosphate buffer, with 2% paraformaldehyde, pH 6.20), through an abdominal cannula. Kidneys slices were immersed in periodate–lysine–paraformaldehyde solution at 4 °C overnight. The tissue was embedded in paraffin for light microscopic immunohistochemical analysis. Paraffin sections were cut. After deparaffining, the sections were incubated with 3% H_2O_2 for 15 min (to eliminate endogenous peroxidase activity) and then with blocking serum for 15 min. The sections were then incubated with polyclonal antibodies against OAT1 or OAT3 overnight. Rabbit polyclonal antibodies were raised against a synthesized polypeptide of the carboxyl terminal of rOAT1 or of rOAT3 [29,30]. The sections were rinsed with PBST and incubated with biotinylated secondary antibody against rabbit Immunoglobulin for 1 h (biotinylated Ig Multi-Link Biogenex). After being rinsed with PBST, the sections were incubated for 30 min with horseradish peroxidase (HRP)-conjugated streptavidin solution (Streptavidin/HRP complex Multi-Link Biogenex). In order to detect HRP labeling, a peroxidase substrate solution with diaminobenzidine (0.05% diaminobenzidine in PBST with 0.05% H_2O_2) was used. The sections were counterstained with hematoxylin before being examined under a light microscopic.

2.7. Na–K-ATPase activity assay

Na–K-ATPase activity was estimated as the difference between the amounts of inorganic phosphate liberated in the absence (total ATPase) and in the presence (Mg^{+2}-ATPase) of ouabain [31]. The release of inorganic phosphate was measured according to Widnell et al. [32].

2.8. Renal excretion studies

These studies were performed as previously described [21,25,33]. Sham ($n=4$) and CRF ($n=5$) rats were anaesthetized as described. Femoral vein and artery were cannulated and a bladder catheter (3 mm i.d.) was inserted through a suprapubic incision. A priming dose of inulin (0.6 mg/kg b.w.) and PAH (30 mg/kg b.w.) in 1 mL of saline solution was administered through the venous catheter. Then, a solution containing inulin (18 g/L), PAH (6 g/L) and saline solution (9 g/L) was infused through the venous catheter employing a constant infusion pump (Pump 22; Harvard Apparatus, USA) at a rate of 1 mL/h/100 g b.w. After equilibrating for 45 min, urine was collected during two 20-min periods. Blood from the femoral artery was obtained at the midpoint of each clearance period. Arterial blood pressure was estimated throughout the experiments with a manometer inserted in the femoral artery. The glomerular filtration rate (GFR) was calculated from the clearance of inulin, in order to determine the filtered load of PAH. The excreted, secreted and filtered loads of PAH were calculated by conventional formulae for each animal. PAH concentrations in the serum and urine were determined by the method of Waugh and Beall [34] and inulin concentrations were assayed by the procedure of Roe [35]. The volume of urine was determined by gravimetry.

2.9. Pharmacokinetic studies

These studies were done similar to previously described [20,25,27]. The day of the experiments, Sham ($n=5$) and CRF ($n=6$) rats were anaesthetized with sodium thiopental (70 mg/kg b.w., i.p.). The femoral artery and the vein were both catheterized to obtain samples and to administer test compound, respectively. A single bolus of PAH (30 mg/kg b.w., aqueous solution, i.v.) was administered.

Table 1
Body weight, kidney weight, kidney/body weight ratio, systolic blood pressure (SBP), and plasma creatinine concentrations (PCr) in Sham and CRF rats

	Sham ($n=5$)	CRF ($n=6$)
Body weight (g)	494 ± 21	494 ± 28
Kidneys weight (g)	3.29 ± 0.12	2.22 ± 0.18*
Kidney/body weight ratio	0.00671 ± 0.00036	0.00446 ± 0.00019*
SBP (mm Hg)	126 ± 2	150 ± 4*
PCr (mg%)	0.94 ± 0.04	2.10 ± 0.02*

Results are expressed as mean values ± S.E.
* $P<0.05$.

Blood samples were obtained at 0–15 min range time after the administration of the PAH solution. The volume of blood samples was 50 μL. Seven blood samples were removed from each rat at different times between 0 and 15 min. An equivalent volume of isotonic saline solution was infused to restore the amount extracted in the blood samples. Samples were centrifuged at 3000 rpm for 3 min, and the extracted plasmas were frozen at −20 °C until analysis.

The plasma concentration vs. time curves for PAH, for each individual animal, were fitted with the PKCALC computer program [36]. Data were fitted to a biexponential curve. The choice of the best fit was based on the determination of coefficient values (R^2) and F test [37,38]. All fits had R^2 values >0.9. The following equation was used to describe the biexponencial concentration–time curves:

$$Cp = Ae^{-\alpha t} + Be^{-\beta t}$$

where Cp is PAH plasma concentration (mg%) at time t (min) after administration; α represents the distribution from the central compartment and β is an equilibrium constant reflecting the dynamics between k_{21} and K_{10} and the slopes of each of the adjusted curves give their values. A and B represent the initial values of the distribution and elimination components, respectively, extrapolated from the y-axis intercept. The estimate parameters (α, β, A, and B) were used to solve the first-order rate constants of transfer from the central to the peripheral compartments (k_{12} and k_{21}) and the elimination rate constant from the central compartment (K_{10}) with classical equations. The derived parameters are: area under curve (AUC), total volume of distribution (VdT), volume of the central compartment (VdC), volume of the peripheral compartment (VdP), steady-state volume of distribution (Vdss), systemic clearance (Cls), elimination half- life ($t_{1/2}$ β) were calculated according to standard procedures for compartmental analysis. Cls was calculated as Dose/AUC. The formulas for calculation of the different volume of distribution were: VdT = Dose / [Beta × AUC], VdC = Dose / A + B, VdP = VdT − VdC, Vdss = Cls × MDRT, MDRT (mean disposition residence time) = AUMC / AUC, AUMC is the area under curve for the plot of the product of concentration and time vs. the time from time zero to infinity. The concentration of PAH in plasma was measured using the method described by Waugh and Beall [34].

2.10. Materials

Chemicals were purchased from Sigma (St. Louis, MO, USA) and were analytical grade pure. Polyclonal antibody against OAT1 and the OAT1 peptide for Western blot was purchased from Alpha Diagnostic International (San Antonio, TX, USA). The polyclonal antibody against OAT1 for immunohistochemical studies and the polyclonal antibody against OAT3 and the OAT3 peptide for both Western and immunohistochemical studies were non-commercial [29,30].

2.11. Statistical analysis

Statistical analysis was performed using an unpaired t-test. When variances were not homogeneous a Welch's

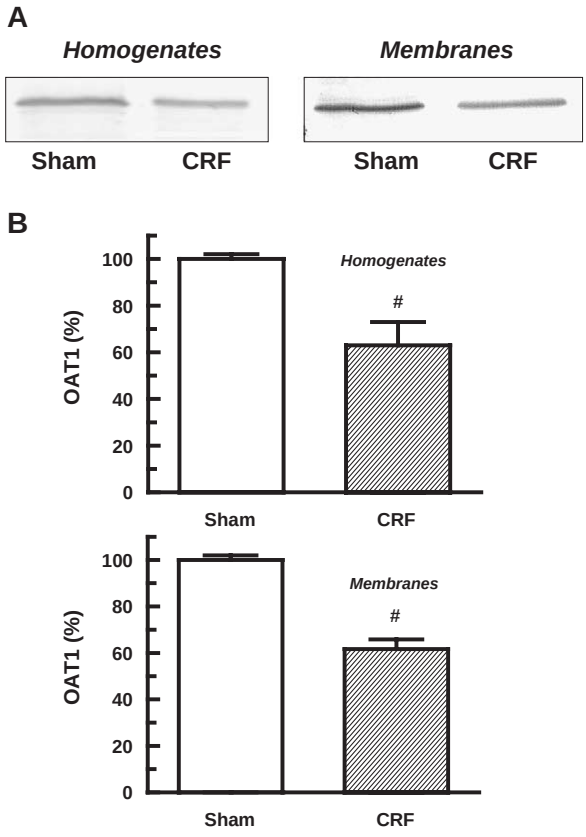

Fig. 1. (A) Renal homogenates (50 μg proteins) and basolateral membranes (50 μg proteins) from kidneys of Sham and CRF rats were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (8.5%) and blotted onto nitrocellulose membranes. OAT1 was identified using commercial polyclonal antibodies as described in Materials and methods. (B) Densitometric quantification of OAT1. Sham levels were set at 100%. Each column represents mean ± S.E. from experiments carried out in triplicate on four different homogenates and basolateral membrane preparations for each experimental group. $^{\#}P<0.05$.

correction was employed. P values less to 0.05 were considered significant. Values are expressed as means ± standard error (S.E.). For these analysis a GraphPad software was used.

3. Results

Body weight, kidney weight, kidney/body weight ratio, SBP and PCr are shown in Table 1. PCr levels rose to around 220% of Sham. Histological studies revealed lymphocitic infiltrate in the interstitium, a moderate dilatation of proximal tubules, decreased cellular height and tubular tiroidization (data not shown).

When kidney homogenates and basolateral plasma membranes from Sham and CRF animals were subjected to immunoblot analysis for OAT1 protein, a primary band with a size of 57 kD was detected. Fig. 1 shows a decrement of approximately 40% both in homogenates and membranes in the expression of OAT1 in CRF rats. The OAT1 protein of 57 kD disappeared when the antibody was preabsorbed to the synthetic antigen peptide (data not shown).

The immunohistochemistry using horseradish peroxidase-conjugated secondary antibody for light microscopy showed the OAT1 label associated with the basolateral plasma membrane domains of proximal tubules in Sham and CRF rats. Fig. 2 shows that OAT1 labeling was decreased in CRF rats compared with Sham ones.

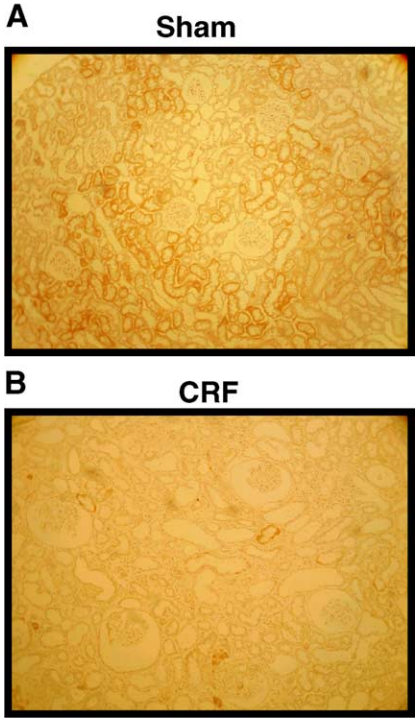

Fig. 2. Immunostaining of OAT1 in kidneys from Sham (A) and CRF rats (B). Serial sections from each rat kidney were stained using a non-commercial anti-rOAT1 antibody (crude immune serum). OAT1 labeling was seen at the basolateral domains of proximal tubule cells. In CRF rats, OAT1 labeling density was much weaker compared with that seen in kidneys from Sham-operated rats. These figures are representatives of typical samples from four rats. Magnification ×150.

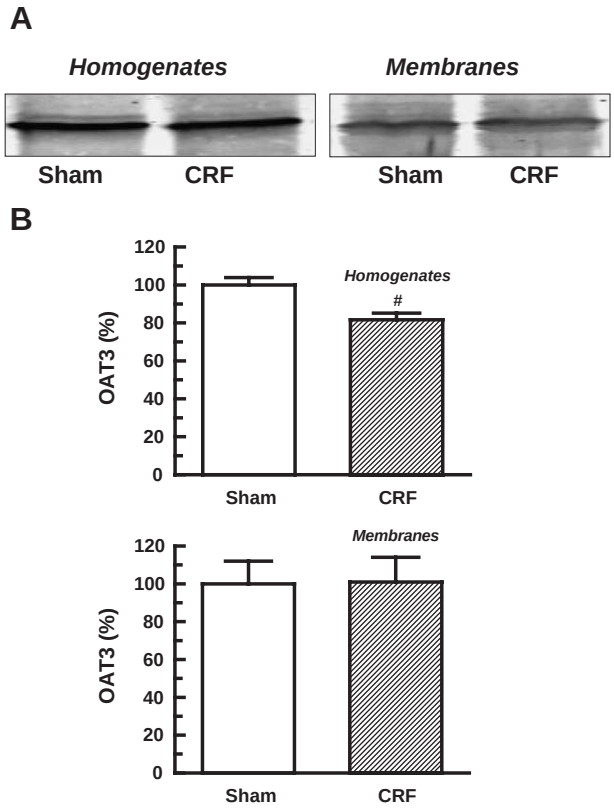

Fig. 3. (A) Renal homogenates (70 μg proteins) and basolateral membranes (70 μg proteins) from kidneys of Sham and CRF rats were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (8.5%) and blotted onto nitrocellulose membranes. OAT3 was identified using non-commercial polyclonal antibodies as described in Materials and methods. (B) Densitometric quantification of OAT3. Sham levels were set at 100%. Each column represents mean ± S.E. from experiments carried out in triplicate on four different homogenates and basolateral membrane preparations for each experimental group. $^{\#}P<0.05$.

Western blots of kidney homogenates and basolateral plasma membranes from Sham and CRF rats showed signals for OAT3 with a protein size of 130 kD. In Fig. 3 it can be observed that OAT3 abundance was slightly decreased in kidney homogenates from CRF rats as compared with Sham ones. The densitometry of these bands revealed a decrease in OAT3 from CRF to 82 ± 3% of sham-operated control levels. No differences were observed for OAT3 expression in basolateral plasma membranes from both experimental groups. These signals were not observed when the antibody was preabsorbed with the OAT3 peptide (data not shown).

The immunoperoxidase microscopy for OAT3 showed no appreciable differences in the staining for both Sham and CRF kidneys (data not shown).

Values of alkaline phosphatase abundance in kidney homogenates were not significantly different between Sham and CRF rats (100 ± 19%, $n=4$, vs. 103 ± 14%, $n=4$).

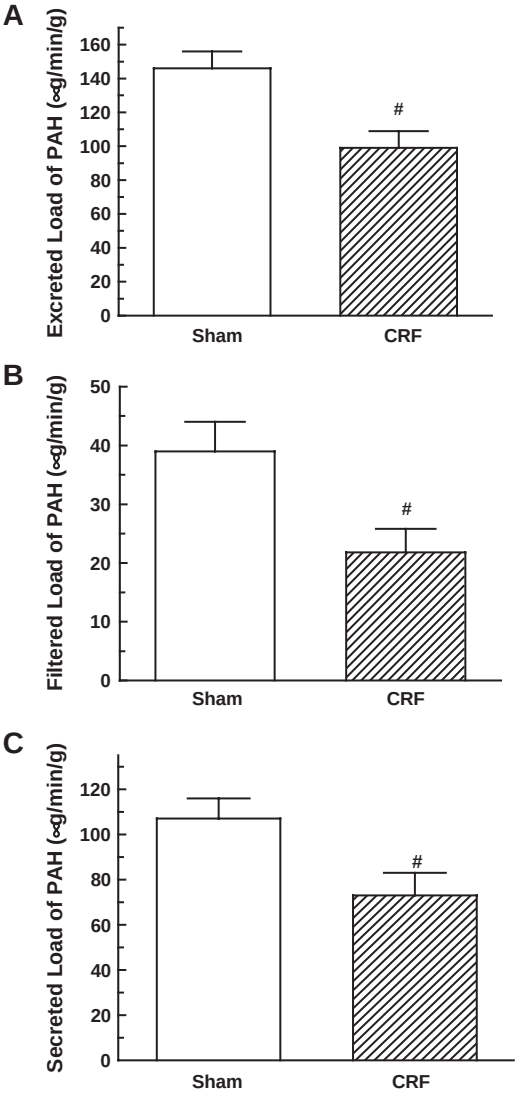

Fig. 4. Excreted (A), filtered (B) and secreted (C) loads of PAH in Sham ($n=4$) and CRF ($n=5$) rats expressed per gram of kidney weight. Results are expressed as means ± S.E. $^{\#}P<0.05$.

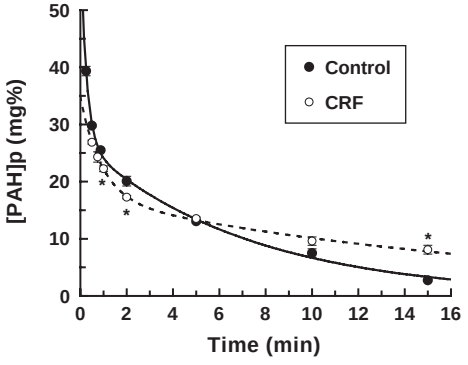

Fig. 5. Mean plasma concentration–time profiles of PAH in Sham ($n=5$) and CRF ($n=6$) rats following a single 30 mg/kg b.w., i.v. dose of PAH. Seven blood samples were taken from each animal at different times between 0 and 15 min. Results are expressed as means ± S.E. $^{*}P<0.05$. S.E. is lower than the size of the symbols for some values.

Table 2
Pharmacokinetic parameters of PAH in Sham and CRF rats after a single dose (30 mg/kg b.w., i.v.)

	Sham ($n=5$)	CRF ($n=6$)
AUC (mg × s/mL)	2.03 ± 0.07	3.80 ± 0.61*
Cls (mL/min/100 g, b.w.)	1.48 ± 0.05	0.885 ± 0.123*
K_{10} (min^{-1})	0.550 ± 0.132	0.108 ± 0.022*
$t_{1/2}$ (β) (min)	4.31 ± 0.14	9.40 ± 1.09*
VdT (mL/100 g, b.w.)	9.82 ± 0.41	16.50 ± 0.81*
VdC (mL/100 g, b.w.)	3.95 ± 1.52	8.85 ± 0.84*
VdP (mL/100 g, b.w.)	5.86 ± 1.28	7.65 ± 0.85
Vdss (mL/100 g, b.w.)	10.22 ± 0.38	15.13 ± 0.48*

Results are expressed as means ± S.E.
AUC=area under curve; Cls=systemic clearance; K_{10}=elimination constant from the central compartment; $t_{1/2}$ (β)=elimination half-life ($t_{1/2}$); VdT=total volume of distribution; VdC=volume of the central compartment; VdP=volume of the peripheral compartment; Vdss=steady-state volume of distribution.
* $P<0.05$.

The heterogeneous changes in the abundance of OAT1, OAT3 and alkaline phosphatase observed in CRF rats underline the selectivity of the response.

Na–K-ATPase activity decreased in kidney homogenates from CRF rats as compared with Sham ones (μmol Pi/h/mg protein, 9.1 ± 0.4, $n=4$, vs. 4.9 ± 0.9, $n=4$, $P<0.05$).

CRF rats showed an important decrease of GFR as compared with Sham rats (μL/min/g, 94 ± 17, $n=5$, vs. 638 ± 76, $n=4$, $P<0.05$). As shown in Fig. 4, the excreted (Fig. 4A), filtered (Fig. 4B) and secreted (Fig. 4C) loads of PAH were diminished in CRF rats. The excreted load of PAH was significantly lower in CRF group as consequence of a diminution of both filtered and secreted loads.

The pharmacokinetic studies revealed that PAH concentration decreases in the central compartment following a biexponencial function as it is shown in Fig. 5. Lower plasma concentrations of PAH in CRF group were observed at first time points. This behavior during the distribution phase might be due to the increment in the VdC observed in CRF rats. On the contrary, higher plasma concentrations of PAH in the CRF group were displayed during the elimination phase (last time points), indicating the impairment in the elimination of this organic anion. The pharmacokinetic parameters of PAH were all statistically different in CRF rats as compared with Sham ones, with the exception of VdP. Lower values of PAH systemic clearance and K_{10} in CRF rats as compared with Sham ones are shown in Table 2. In addition to the statistically significant increase in VdC, CRF rats also showed higher values of VdT and Vdss. The Vdss was used to evaluate the differences in drug distribution, since changes in VdT are also associated with changes in drug elimination [38–40].

4. Discussion

Several studies have focused in the mechanism by which renal function declines in chronic renal failure following

subtotal renal ablation but it still remains unclear. Some of them have examined the importance of glomerular changes in the initiation and progression of renal damage [1]. However, it seems that tubular damage is at least as important as glomerulosclerosis. Tubular activity and function have been little examined in CRF. Few data are available on the changes in the activity of tubular cell membranes where important transporters and energy regulators reside [5–7,16,17].

Renal impairment is closely associated with increased uremic toxicity which is characterized by accumulation of end products of protein metabolism and various hormonal peptides [41].

The dosage regimen of drugs must be modified in renal impairment because this failure has a variety of influences on pharmacokinetics and pharmacodynamics [42,43].

The mammalian renal proximal tubule plays an important function by rapidly clearing the blood of endogenous toxins and xenobiotics. In this regard, the renal organic anions secretory pathway has evolved to mediate the excretion of a wide array of negatively charged organic compounds, and PAH has become the model substrate used to functionally characterize the luminal and basolateral membrane transport mechanisms [8]. OAT1 and OAT3 represent the main basolateral polyspecific transporters for organic anions [8–11,44]. Little is known about the modulation of these transporters, especially about OAT1 and OAT3 regulatory aspects concerning the body's response to disease states. In our laboratory we have demonstrated modifications in the expression of OAT1 in kidneys from rats with different pathologies such as: arterial calcinosis [21], extrahepatic cholestasis [27] and ureteral obstruction [45].

In the present study we performed in rats with CRF, induced by means of 5/6 nephrectomy, semiquantitative immunoblotting and immunohistochemistry for OAT1 and OAT3, PAH pharmacokinetics and PAH renal excretion with the following purposes: (1) to examine whether there are changes in renal OAT1 and OAT3 expression and (2) to examine whether these changes are associated with changes in organic anions excretion.

Our data revealed that CRF rats have a lower expression of OAT1 protein in both kidney homogenates and renal basolateral membranes. These results were confirmed by immunohistochemistry. On the other hand, a slightly decrease in OAT3 expression was observed in homogenates from CRF kidneys that was not evidenced in immunohistochemistry studies. No modifications in OAT3 abundance were evidenced in renal basolateral plasma membranes from CRF group. The specificity of these alterations were also demonstrated by the lack of changes in the alkaline phosphatase protein expression in renal homogenates.

We evaluated the excretion of PAH in Sham and CRF rats. Interestingly, this parameter was diminished in CRF rats. GFR and consequently the filtered load of PAH were also lower in CRF rats as compared with Sham ones. Moreover, the secreted load of PAH was diminished in the CRF group. Therefore, the differences observed in renal excretion of PAH are accounted for variations in filtered and secreted load of this organic anion. The decrease in the renal excretion of PAH observed in CRF rats was evidenced in pharmacokinetic parameters of PAH such as K_{10} and Cls.

The medium PAH plasma concentrations reached during the renal clearance infusion studies were 314 ± 28 and 1191 ± 162 μM for Sham and CRF rats respectively. The OAT1 and OAT3 mediated uptake of PAH is saturable with apparent Michaelis constants ranging 15 to 70 μM for rat OAT1 [9,10] and 60 to 90 μM for rat OAT3 [8,11]. So, the PAH concentrations that we obtained in our "in vivo" experiments were sufficiently high above the reported Km of rat OAT1 and OAT3. The diminished secreted load of PAH measured under saturating conditions might be in part account for the lower number of OAT1 protein units observed in basolateral plasma membranes from CRF kidneys. In addition, the transport of organic anions mediated by OAT1 and OAT3 occurs by indirect coupling of the sodium gradient, and this gradient is generated by Na,K-ATPase pump. The present study shows that the Na,K-ATPase pump activity was reduced in CRF rats. In this connection, Kwon et al. [7] have reported that there is significant decrease in total kidney levels of Na,K-ATPase in rats with CRF. Moreover, Maxild et al. [46] have demonstrated that the inhibition of aerobic accumulation of PAH by ouabain in cortex slices of rabbit kidney is related to a decrease in Na,K-ATPase activity.

Contrasting with OAT1 down regulation, MRP2 upregulation in CRF rats has been described by Laouari et al. [16]. Therefore, the protein expression of the luminal (MRP2) and basolateral (OAT1) PAH transporters are differently modified in CRF. As highly accumulated anionic drugs may cause further deterioration of renal failure, the molecular mechanism(s) involved in the differential regulation of MRP2 and OAT1 expression should be elucidated to prevent drug-induced toxicity under CRF. Tubular secretion is a vectorial transcelular transport system consisting of basolateral entry into the epithelial cells and efflux across the brush border membranes. Defects in either of these two processes should influence the tubular secretion of ionic drugs. In the present study, the tubular secretion of PAH was markedly reduced, corroborating the concept that the limiting step for the urinary excretion of PAH is the tubular secretion step across the basolateral membranes.

It is also important to mention that there might be other factors involved in the reduced PAH renal excretion in CRF such as the reduction in renal blood flow [1] that limits the delivery of the drug to the OAT1 and the competition for peritubular uptake [8,47] and for luminal secretion [48] with other organic anions that include urate

[9] that accumulate in uremia. Metabolic acidosis, that is another characteristic feature of CRF, depolarizes the membrane potential of proximal tubule cells [49] decreasing consequently organic anions secretion. The increased VdT as consequence of the increment in VdC that we observed in CRF rats might also contribute to decrease renal PAH delivery. The increase in this VdC might be due to the fact that animals with CRF have a 10 to 30% increment in extracellular and blood volume [50].

CRF is well known to be associated with elevated serum parathyroid hormone (PTH) levels and changes in mineral ion homeostasis including secondary hyperparathyrodism [1]. Nagai and co-workers [51] have showed an inhibition of basolateral uptake and secretion of organic anions in opossum kidney (OK) cells by parathyroid hormone via a staurosporine sensitive mechanism. The inhibition of basolateral OAT1 by stimulation of protein kinase C (PKC) has been reported in isolated tubules of killifish [52]. Recently, You and co-workers [53] have showed that PKC inhibits murine OAT1 without direct phosphorylation of the transport protein itself. Since PTH is a potent inhibitor of organic anion transport by PKC we speculate that the decreased density and kidney levels of OAT1 in rats with CRF in the present study may be at least partly attributed to the increased levels of serum PTH.

OAT1 also transports diuretics [47]. Its reduced expression might also explain the manifest diuretic resistance in patients with CRF [54]. It is well known that diuretics achieve their site of action after being secreted in the tubular lumen by means of the organic anions transport system. These data would throw new light on mechanisms that contribute to diuretic resistance in models or patients with CRF.

In conclusion, the goal of this study was to evaluate for the first time OAT1 and OAT3 expression in severe CRF. This study demonstrates the functional and molecular alterations of the urinary excretion mechanisms of organic anions in 5/6 nephrectomized rats. The reduction in the protein expression of OAT1 (approximately 40%) or in the activity of Na,K-ATPase pump (approximately 45%) or both would be, at least in part, associated with the organic anions secretion impairment in CRF, which substantially alters the renal capacity for eliminating these compounds and accounts for some accumulated uremic toxins found in CRF.

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