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Effects of Chronic Renal Failure on Liver Drug Transporters

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ABSTRACT:

Chronic renal failure (CRF) is associated with a decrease in liver drug metabolism, particularly mediated by the cytochrome P450. CRF also impedes intestinal drug transporters [mainly P-glycoprotein (P-gp) and multidrug resistance protein (MRP)]. However, very few studies have evaluated the effects of CRF on liver drug transport. The present study aimed to investigate the repercussions of CRF on liver drug transporters involved in hepatic uptake [organic anion transporting polypeptide (Oatp) 2] and in drug extrusion (P-gp and MRP2). Two groups of rats were studied: control and CRF. Oatp2, P-gp, and MRP2 protein expressions and mRNA levels, as well as some of their metabolic activity, were assessed. The effects of CRF serum on drug transporters were also evaluated in cultured hepatocytes. Compared with control, creatinine clearance was reduced by 70% ($p < 0.01$) in rats with CRF. Protein expression and mRNA levels of P-gp were increased by 25 and 40% ($p < 0.01$), respectively, in liver from rats with CRF. MRP2 protein expression was identical in both groups, whereas its mRNA levels were increased by 35% ($p < 0.01$) in CRF rats. Finally, Oatp2 protein expression was reduced by 35%, whereas its mRNA levels remained unchanged. Similar results were obtained when hepatocytes were incubated with uremic serum. In conclusion, CRF is associated with a decrease in liver transporters involved in drug absorption and an increase in those involved in drug extrusion. Uremic mediators appear to be responsible for these modifications.

Several studies have shown that the metabolic clearance of various substrates is reduced in patients with chronic renal failure (CRF) (Touchette and Slaughter, 1991; Talbert, 1994; Matzke and Frye, 1997). The decrease in drug biotransformation is mainly secondary to alterations in drug metabolism by the liver (Touchette and Slaughter, 1991). Indeed, in rats with experimental renal failure, studies have shown that CRF induces a marked decrease in liver cytochrome P450 (P450) activity secondary to reduced protein and gene expression of selective P450 isoforms, namely, CYP2C11, CYP3A1, and CYP3A2 (Leblond et al., 2001). This down-regulation is produced by uremic factors, namely, parathyroid hormone (PTH).

Another major route for drug elimination is drug extrusion by transporters. These membranous proteins are located in several epithelia, such as the kidney, the liver, and the intestine. They are implicated not only in renal, biliary, or intestinal drug extrusion but also in their uptake, especially in enterocytes and hepatocytes. Recently, we have shown that CRF could impede several drug transporters in the intestine (Naud et al., 2007). Indeed, P-glycoprotein (P-gp) (also known as mdr1a, *abcb1* gene family) and multidrug resistance-related protein (MRP) type 2 (also known as cMOAT, *abcc2* gene family) are decreased significantly in the intestine of CRF rats compared with control animals. Organic anion transporting polypeptide (Oatp) type 3 (gene *Slc21a7*) was not modified by uremia. These findings could explain, in part, why patients with CRF have an increase in bioavailability of some drugs (Naud et al., 2007).

Several drug transporters are present in the hepatocytes. Uptake transporters are mainly located on the basolateral membrane of the hepatocytes and mediate drug uptake from the blood circulation to the hepatocytes. Excretion transporters are mainly located on the apical membrane of the hepatocytes and mediate excretion of the drugs from the hepatocytes to the biliary canaliculi. Most common uptake transporters include Oatp, organic cation transporters, organic anion transporters, and sodium-dependent hepatocyte bile-salt uptake system, whereas most common excretion transporters include hepatocellular bile-salt export pump, breast cancer resistance protein, MRP, and P-gp (Meier and Stieger, 2002; Takikawa, 2002; Kullak-Ublick et al., 2004).

Very few studies have evaluated the repercussions of CRF on liver drug transporters, and conflicting results have been published. Laouari et al. (2001) have reported that in CRF rats MRP2 was increased whereas P-gp was unchanged compared with control animals. However, the severity of renal failure was quite low. This could have confounded the results because it is well established that there is a strong correlation between the severity of CRF and the modulation of metabolizing enzymes (both in the liver and intestine), as well as in drug transporters (in the intestine) (Pichette and Leblond, 2003; Naud et al., 2007). Furthermore, they did not study the uptake transporters. On the other hand, Sun et al. (2004) have shown that some uremic

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ABBREVIATIONS: CRF, chronic renal failure; P450, cytochrome P450; PTH, parathyroid hormone; P-gp, P-glycoprotein; MRP, multidrug resistance-related protein; Oatp, organic anion transporting polypeptide; PBS, phosphate-buffered saline; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PCR, polymerase chain reaction; HPLC, high-performance liquid chromatography.

toxins present in the serum of CRF patients decrease the uptake of erythromycin in hepatocytes, suggesting an inhibition of Oatp2.

The objectives of this study were to determine the effects of CRF on hepatic drug transporters. For this purpose, we measured in control and CRF rats 1) liver protein and gene expression of P-gp (mdr1a, *abcb1*), MRP2 (cMOAT, *abcc2*), and Oatp2 (gene *Slc21a5*), and 2) in vivo activity of P-gp. Furthermore, we also evaluated the effect of uremic serum on the drug transporters in cultured hepatocytes.

Materials and Methods

Experimental Model. Male Sprague-Dawley rats (Charles River Canada, Montreal, QC, Canada), weighing 200 to 300 g were housed in the Research Center animal care facility and maintained on Harlan Teklad rodent diet (Harlan Teklad Global Diets, Montreal, QC, Canada) and water ad libitum. An acclimatization period of at least 3 days was allowed to the animals before any experimental work was undertaken. All the experiments were conducted according to the Canadian Council on Animal Care guidelines for care and use of laboratory animals and under the supervision of our local animal care committee.

Experimental Protocol. Studies were performed in two groups of 35 animals each: control pair-fed and CRF. Hepatic drug transporters' protein expression and mRNA levels were measured in 28 rats/group. P-gp activity was measured in vivo using clearance of rhodamine 123, a P-gp substrate, in 7 rats/group.

CRF was induced by two-stage five-sixth nephrectomy as previously described (Leblond et al., 2001). Control pair-fed rats were fed the same amount as CRF rats ate on the previous day and water ad libitum to control for the effect of CRF-induced malnutrition. At day 41 after the nephrectomy, the rats were housed in metabolic cages, and urine was collected for 24 h to determine the clearance of creatinine. Rats were sacrificed by decapitation 42 days after nephrectomy. Blood was collected for the measurement of serum creatinine and urea.

Preparation of Liver Homogenates. Rat liver was immediately excised following sacrifice, rinsed in ice-cold saline, and flash-frozen in liquid nitrogen. Samples were stored at −80°C until homogenate preparation. Briefly, 200 mg of liver was homogenized in 1 ml of 0.9% NaCl, 0.1 mM phenylmethylsulfonyl fluoride using a Potter-Elvehjem tissue grinder (Wheaton Science Products, Millville, NJ). Samples were then sonicated on ice for 10 s and filtered across a 5-μm membrane (PALL, St. Laurent, QC, Canada), and aliquots were stored at −80°C up to analysis.

Hepatocyte Isolation and Culture. The hepatocytes were isolated from male Sprague-Dawley rats weighing 200 to 250 g and cultured as previously described (Guevin et al., 2002; Michaud et al., 2005). Hepatocytes were incubated for 48 h in culture medium containing 10% rat control or CRF serum. Hepatocytes were harvested by scraping in phosphate-buffered saline (PBS) with 0.1 mM phenylmethylsulfonyl fluoride for protein analysis and in RLT buffer (Qiagen, Mississauga, ON, Canada) for mRNA analysis. Samples were stored at −80°C up to analysis.

In Vivo Excretion of Rhodamine. To evaluate the in vivo activity of P-gp in the liver of CRF and control rats, biliary transport of rhodamine 123 was evaluated on anesthetized rats using an adaptation of the protocol published by Yumoto et al. (1999). Briefly, the right femoral vein was catheterized with polyethylene tubing, and a 1-ml bolus of saline (0.9% NaCl) was administered, followed by perfusion at 2 ml/h for a 30-min stabilization period. Meanwhile, the left femoral artery, the bile duct, and the urinary bladder were cannulated for blood, bile, and urine collection. After the stabilization period, a bolus of 4.36 ml/kg rhodamine 123 (Sigma, Oakville, ON, Canada) was injected at a concentration of 100 μM in PBS, followed by perfusion at 2 ml/h for a 50-min stabilization period, at the end of which collection began. Total bile and urine were collected every 10 min for 40 min, and 200 μl of blood was collected halfway through each collection period under constant rhodamine 123 perfusion. A 2.7-ml/kg bolus of cyclosporine A (4.16 mM), a P-gp inhibitor, was then administered, followed by perfusion (2 ml/h) of rhodamine 123 (100 μM) and cyclosporine A (80 μM) in PBS for a 20-min stabilization period. Finally, bile, urine, and blood were collected for 50 min under constant perfusion. At the end of the protocol, animals were sacrificed, and the liver was excised, flash-frozen in liquid nitrogen, and kept at −80°C until analysis.

Western Blot Analysis. Three major hepatic transporters were assessed by Western blot analysis following a previously described protocol (Leblond et al., 2002; Naud et al., 2007): P-gp, MRP2, and Oatp2. Each blot was repeated three times, and results were pooled to obtain reported values. Protein expression in control animals was arbitrarily defined as 100%.

TABLE 1

Nucleotide sequences of PCR primers

Gene	Primer Sequence (5′-3′)	Predicted Product Size
		base pair
mdr1a	Sens: ATCAACTCGCAAAAGCATCC	116
	Anti-sens: AATTCAACTTCAGGATCCGC	
mrp2	Sens: GCTGGTTGGAAACTTGGTCG	93
	Anti-sens: CAACTGCCACAATGTTGGTC	
oatp2	Sens: TGTGATGACCTGTGATAATTTTCCA	81
	Anti-sens: TTCTCCACATATAGTTGGTGCTGAA	
Gapdh	Sens: TAAAGGGCATCCTGGGCTACACT	200
	Anti-sens: TTACTCCTTGGAGGCCATGTAGG	

Mdr1a, *mrp2*, and *gapdh* primers were designed based on published cDNA sequences with the help of the Jellyfish and BLASTIN 2.20.1 computer programs (Altschul et al., 1997). Oatp2 sequence was as previously published by Gao et al. (2004). The resulting PCR products were sequenced on an ABI Prism 3100 analyzer (Applied Biosystems, Foster City, CA).

P-gp and MRP2 were detected using monoclonal antibodies from ID Labs (London, ON, Canada): C219 and M_2 III-6, respectively. Oatp2 was detected using a rabbit anti-rat Oatp2 antibody from US Biological (Swampscott, MA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), used as a loading control, was detected using rabbit anti-GAPDH from Abcam (Cambridge, MA). In preliminary experiments, we have shown that GAPDH expression is not modulated by CRF compared with controls (data not shown).

mRNA Analysis. At the time of sacrifice, liver was rinsed in ice-cold saline and flash-frozen in liquid nitrogen. Samples were kept at −80°C until RNA extraction. Total RNA was extracted from frozen tissue by the RNeasy mini-kit (Qiagen). RNA concentrations were determined by measuring absorbance at a wavelength of 260 nm. One microgram of total RNA was used to prepare cDNA by reverse transcription using Omniscript RT kit from Qiagen and random primer from Invitrogen (Burlington, ON, Canada). The mRNA encoding for P-gp (mdr1a), MRP2, Oatp2, and GAPDH was measured by quantitative real-time polymerase chain reaction (PCR) using Platinum SybrGreen PCR Supermix UDG from Invitrogen and appropriate primers on an Icycler thermocycler (BioRad, Mississauga, ON, Canada) with 30-s incubation at 94°C, 30-s incubation at 60°C, and 30-s incubation at 72°C. Table 1 shows the sequences of primers used for the quantification of mRNA for each transporter. PCR products were analyzed using the $\Delta\Delta C_T$ method (Livak and Schmittgen, 2001) using GAPDH as standard gene.

Rhodamine 123 Extraction and High-Performance Liquid Chromatography Analysis. Rhodamine was isolated by solid-phase extraction using Sep-Pack Vac 1cc C18 Cartridges (Waters, Milford, MA). Briefly, columns were primed with 100% methanol, washed with water, and acidified samples (20 μl of phosphoric acid/ml sample) were loaded. Columns were then washed with 5% methanol, and the samples were eluted in 100% methanol. Samples were evaporated under nitrogen stream and resuspended to their initial volume in mobile phase (acetic acid 1% and acetonitrile, 60:40, %v/v) for high-performance liquid chromatography (HPLC) analysis. HPLC was conducted as described by Yumoto et al. (1999) on a Beckman System Gold HPLC (Beckman Coulter, Mississauga, ON, Canada) with a Jasco FP-920 fluorescence detector (Jasco, Tokyo, Japan) at 485-nm excitation and 546-nm emission wavelengths on a Gemini 5-μm C18 110Å column (Phenomenex, Torrance, CA). Sample concentration was obtained by interpolation on a standard curve made with pure rhodamine 123 treated by the same procedure.

Rhodamine Excretion Analysis. Biliary clearance was determined using the equation $Cl_b = (C_b \times V_b)/(C_{p,ss} \times \text{time})$ where C_b is the rhodamine concentration in the bile, V_b is the bile volume, and $C_{p,ss}$ is the rhodamine concentration in the plasma at steady state. Urinary clearance was determined using the equation $Cl_u = (C_u \times V_u)/(C_{p,ss} \times \text{time})$ where C_u is the rhodamine concentration in the urine and V_u is the urine volume.

Other Assays. Blood and urine chemistries were determined with an Hitachi 717 autoanalyzer (Roche Diagnostics, Basel, Switzerland).

Statistical Analysis. The results are expressed as mean ± S.E.M. Differences between groups were assessed by using an unpaired Student's *t* test or an analysis of variance test. Significant analysis of variance was followed by a Scheffé post hoc comparison of groups. The threshold of significance was $p < 0.05$.

TABLE 2

Characteristics of control and CRF rats

	Control (n = 35)	CRF (n = 35)	p Value
Body weight (g)	359.1 ± 6.1	322.0 ± 10.3	<0.01
Serum creatinine (μM)	52.5 ± 0.9	189.8 ± 18.8	<0.001
Creatinine clearance (μl/100 g b.wt./min)	323.2 ± 12.2	82.1 ± 8.3	<0.001
Serum urea (mM)	5.0 ± 0.2	40.1 ± 5.8	<0.001

Data are the mean ± S.E.M. Measurements were made at the time of sacrifice. Urinary collection was begun the day before. Blood urea and creatinine and urine creatinine were determined with an Hitachi 717 autoanalyzer (Roche Diagnostics, Basel, Switzerland).

Results

Biochemical Parameters and Body Weight in Control and CRF Rats. Table 2 presents the biochemicals and body weight of the two groups of animals studied. Compared with control pair-fed animals, CRF rats had higher levels of plasma creatinine and lower values of creatinine clearance (reduced by 75%; $p < 0.001$).

Protein Expression of Hepatic Transporters in Control and CRF Rats. As shown on Fig. 1, P-gp was induced by 25% ($p < 0.001$) in CRF rats compared with controls, whereas another excretion transporter, MRP2, remained stable. On the other hand, the import transporter Oatp2 was significantly reduced by 40%. GAPDH, used as a loading control, was not affected by CRF (data not shown). We found a significant correlation between P-gp and Oatp2 protein expression and creatinine clearance ($r = 0.303$, $p < 0.001$ and $r = 0.524$, $p < 0.001$, respectively; data not shown).

mRNA Encoding Hepatic Transporters in Control and CRF Rats. Figure 2 presents the results of the analysis of mRNA coding for liver transporters. There was a significant increase in mRNA for the excretion transporters P-gp and MRP2 (50 and 40%, respectively; $p < 0.05$), but mRNA expression for the import transporter Oatp2 remained unchanged.

In Vivo Excretion of Rhodamine 123 in Control and CRF Rats. To determine the repercussion of P-gp increase on the excretion of drugs, we evaluated the in vivo disposition of rhodamine 123 in control and CRF rats. As shown in Fig. 3, the biliary clearance of rhodamine was significantly increased in CRF rats compared with controls before the addition of the P-gp inhibitor cyclosporine A. However, inhibition of P-gp with cyclosporine caused a significant decrease in the clearance of rhodamine 123 in both groups, bringing them to the same level. The urinary clearance of rhodamine, on the other hand, was significantly decreased in CRF rats (almost 100%).

Protein and mRNA Expression of Transporters in Hepatocytes Incubated with Uremic Serum for 48 h. To determine whether a circulating factor present in the serum of rats with CRF was responsible for the modulation of hepatic transporters, we incubated normal rat hepatocytes with serum from control or CRF rats for 48 h (Fig. 4). Western blot analysis of the hepatocytes showed a significant increase (34%, $p < 0.01$) in P-gp protein expression and a significant decrease (25%, $p < 0.05$) in Oatp2 protein expression in the hepatocytes incubated with serum from CRF rats. On the other hand, MRP2 remained unchanged as observed in vivo. No significant changes were observed in mRNA levels for any of the transporters as opposed to what has been observed in vivo (data not shown).

Discussion

The effects of CRF on the nonrenal elimination of drugs have been studied for many years. It has been well established that CRF causes a decrease in drug metabolism because of a decrease in liver and intestinal P450 expression and activity (Pichette and Leblond, 2003; Sun et al., 2006). A decrease in nonrenal drug elimination could also be attributed to a decrease in phase II elimination or in the excretion by hepatic transporters. In this study, we evaluated the effects of CRF on the hepatic expression of two excretion transporters (P-gp and MRP2) and of one uptake transporter (Oatp2), as well as its effects on P-gp activity.

Our results show that CRF causes a significant 25% increase in P-gp protein expression probably because of the 50% increase in P-gp mRNA levels. On the other hand, although we found a significant 40% increase in MRP2 mRNA expression, its protein expression was not affected by CRF. Finally, CRF causes a decrease in Oatp2 protein expression, which is not associated with a decrease in mRNA levels. Similar results were obtained when we incubated normal rat hepatocytes with serum from rats with CRF for 48 h. We also evaluated hepatic P-gp activity by measuring the biliary clearance of rhodamine in anesthetized rats. We found a 60% significant increase in the excretion of rhodamine that can be inhibited by cyclosporine A, a

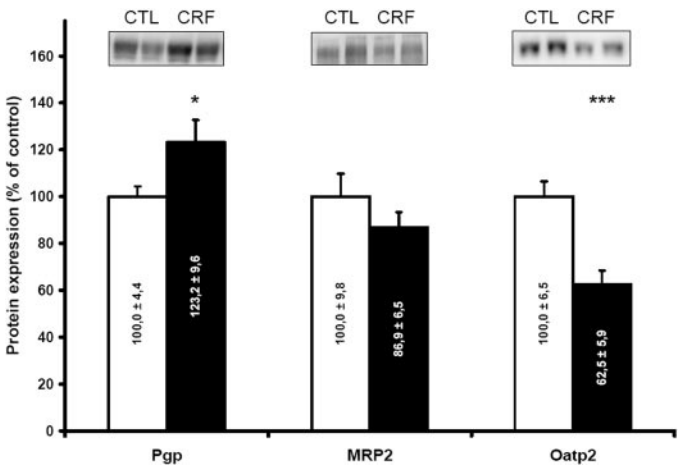

FIG. 1. Protein expression of hepatic transporters in whole liver homogenate from control (white bars) and CRF rats (black bars). Protein bands are expressed in densitometry units. The densitometry units measured for hepatic transporters were standardized by dividing them by the value obtained for GAPDH. The standardized densitometry units of control rats were arbitrarily defined as 100%. Representative blots for each transporter are shown in insert. Data are the mean ± S.E.M. of 28 experiments in each group. *, $p < 0.05$; ***, $p < 0.001$ as compared with control.

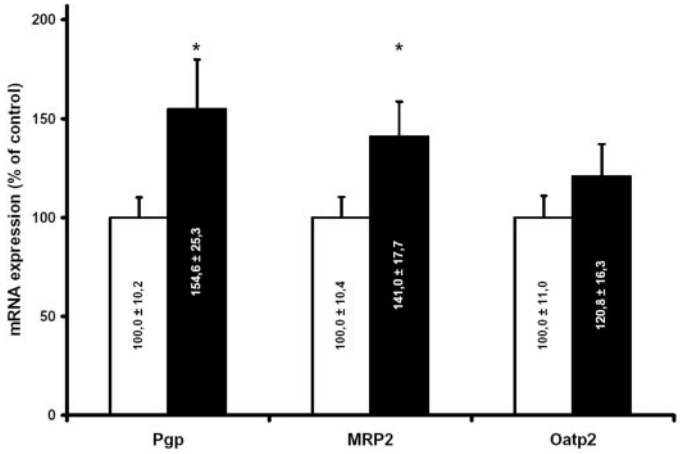

FIG. 2. mRNA encoding hepatic drug transporters in liver from control (white bars) and CRF rats (black bars) was measured by quantitative real-time PCR. mRNA levels are expressed in relative quantities using the $\Delta\Delta C_T$ method (Livak and Schmittgen, 2001) with GAPDH as a standard gene. The number of copies of control rats was arbitrarily defined as 100%. Data are the mean ± S.E.M. of 28 experiments in each group. *, $p < 0.05$ as compared with control rats.

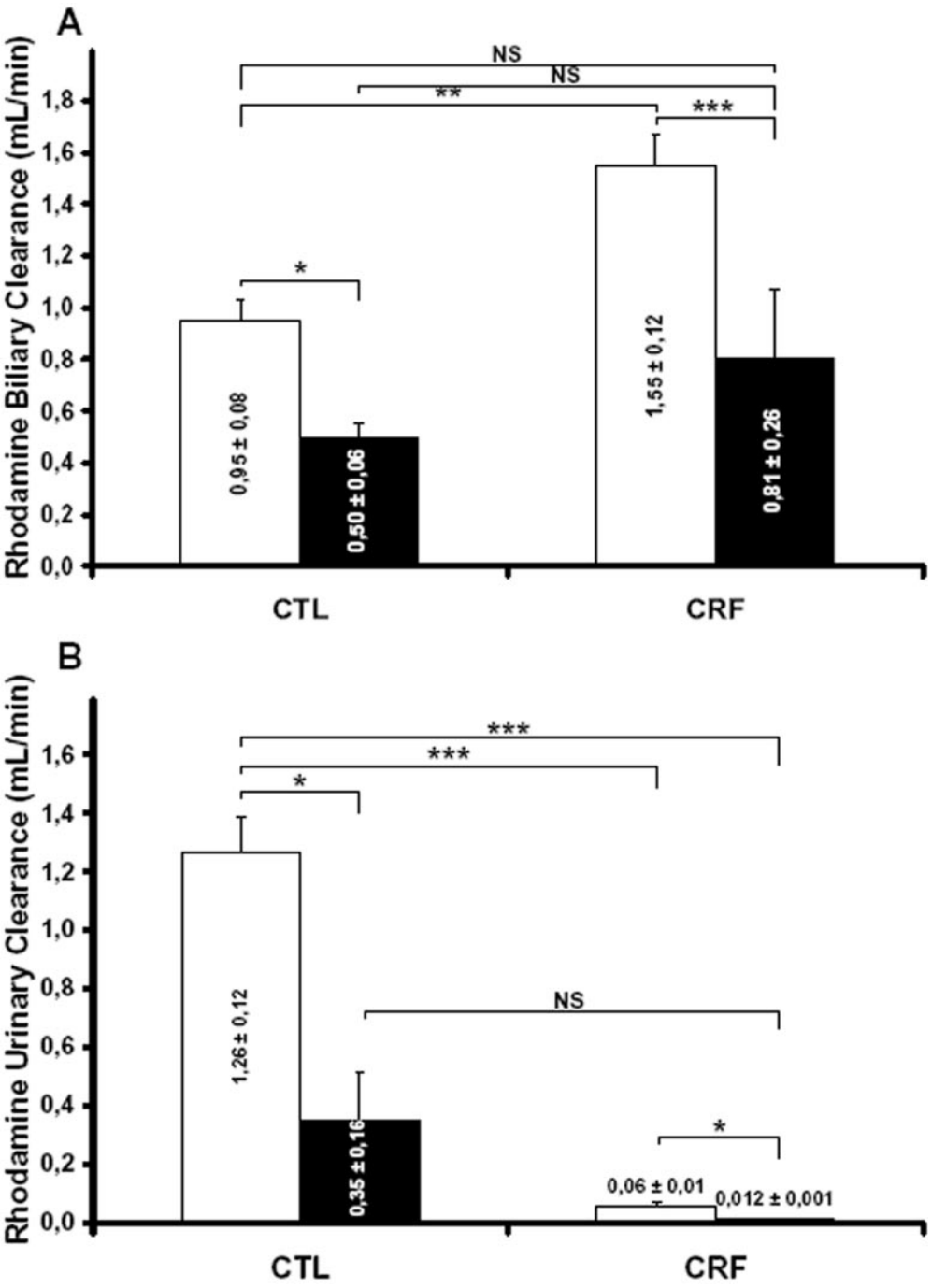

FIG. 3. In vivo excretion of rhodamine 123 in control and CRF rats. A, biliary excretion of rhodamine 123 in anesthetized rats infused with rhodamine 123 in the absence (white bars) and presence (black bars) of the P-gp inhibitor cyclosporine A. Biliary clearance was determined using the equation $Cl_b = (C_b \times V_b)/(C_{p,ss} \times \text{time})$, where C_b is the rhodamine concentration in the bile, V_b is the bile volume, and $C_{p,ss}$ is the rhodamine concentration in the plasma at steady state. B, urinary excretion of rhodamine 123 in anesthetized rats in the absence (white bars) and presence (black bars) of the P-gp inhibitor cyclosporine A. Urinary clearance was determined using the equation $Cl_u = (C_u \times V_u)/(C_{p,ss} \times \text{time})$, where C_u is the rhodamine concentration in the urine and V_u is the urine volume. Data are the mean ± S.E.M. of seven experiments. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$; NS = no statistical difference.

P-gp inhibitor, suggesting that the up-regulation of P-gp protein expression is associated with an increase in activity.

Very few studies have evaluated the effect of CRF on liver drug transporter. A study previously published by Laouari et al. (2001) showed a significant increase in liver MRP2 protein and mRNA expression 6 weeks after nephrectomy was performed on rats. Our results confirmed the increase in MRP2 mRNA levels but not on the protein expression, which could be explained by the specificity of antibodies used. On the other hand, Laouari et al. (2001) also found no changes in P-gp protein and mRNA in the liver of nephrectomized rats, whereas we found a significant increase. The explanation for these conflicting results is not clear. It could be attributed to the degree of severity of the CRF. In their study, nephrectomized rats presented a 60% decrease in creatinine clearance, whereas our rats have a 75% decrease in creatinine clearance. Indeed, we found a significant correlation between the severity of the renal failure as measured by the creatinine clearance and the up-regulation in P-gp, suggesting that, as CRF worsens, P-gp increases.

Our results also show that CRF affects the hepatic transporters in different ways. We have shown a decrease in the expression of the uptake transporter Oatp2 and an increase in the expression and activity of the excretion transporter P-gp. Those transporters work together to eliminate substrates into the bile. Because uptake by Oatp transporters is the first step in the elimination of many xenobiotics, making the compounds available to the detoxification enzymes and the extrusion transporters, and can be a rate-limiting step (Yamazaki et al., 1996; Sun et al., 2006), the inhibition of Oatp2 we found in rats suffering from CRF can probably explain the decrease in drug elim-

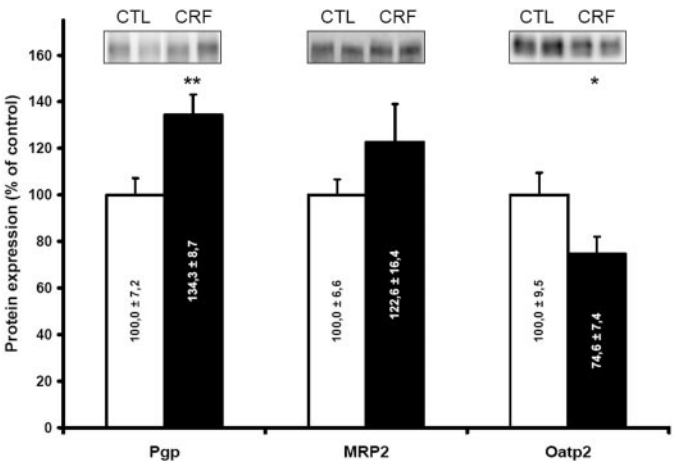

FIG. 4. Level of P-gp, MRP2, and Oatp2 protein expression in hepatocytes incubated with 10% serum from control (white bars) and CRF rats (black bars) for 48 h. Protein bands are expressed in standardized densitometry units (see Fig. 1). Data are the mean ± S.E.M. of 12 experiments. Representative blots for each transporter are shown in insert. No significant changes were observed in mRNA levels in any group (data not shown). *, $p < 0.05$, **, $p < 0.01$ as compared with hepatocytes incubated with serum from control rats.

ination by the liver, as suggested also by Sun et al. (2004, 2006). The increase in P-gp expression and activity could be an adaptive response to the large quantities of organic anions circulating in CRF.

Our results also differ from those we previously published concerning intestinal transporters in CRF rats (Naud et al., 2007). We had found significant decreases in intestinal P-gp and MRP2 protein expression associated with a decrease in their transport activity across the intestinal membrane. We attributed the protein expression decrease to a post-translational mechanism because we found no changes in their mRNA expression. Also, we had found no significant change in Oatp2 and Oatp3 protein and mRNA levels in the intestine of rats with CRF (Naud et al., 2007).

Thus, it appears that CRF affects the expression of drug transporters differently in the main elimination organs, i.e., liver and intestine. However, in both organs, we have shown that the changes in protein and/or mRNA levels for the transporters were caused by a circulating factor present in the serum of rats with CRF. Indeed, we have shown that incubating uremic serum with enterocytes or Caco-2 cells leads to a decrease in P-gp expression and function (Naud et al., 2007). Similarly, in the present study, we showed that uremic serum also modifies the expression of drug transporters in the hepatocytes (Fig. 4). The different potential factors include proinflammatory cytokines, PTH, and uremic toxins, all of which are highly increased in CRF. Interestingly, the proinflammatory cytokine tumor necrosis factor-α is known to increase P-gp protein and mRNA expression and P-gp activity in cultured rat hepatocytes in a time- and dose-dependent manner (Hirsch-Ernst et al., 1998). It is also known to decrease rhodamine 123 transport in Caco-2 cells, a colon carcinoma cell line (Belliard et al., 2004), making it a prime candidate to be responsible of the observed effects of CRF on drug transporters. Sun et al. (2004) have also shown that the uremic toxin 3-carboxy-4-methyl-5-propyl-2-furan-propanoic acid can directly inhibit erythromycin uptake via a decrease in Oatp2 protein expression and activity.

Despite pair-feeding of the rats, we still observed a slight difference (<10%) in body weight between the CRF and control pair-fed rats. Very few studies have evaluated the effects of malnutrition on hepatic transporters. Lee et al. (2003) showed that severe protein and caloric malnutrition caused a decrease in protein expression and activity of liver P-gp. Furthermore, two studies showed that fasting can cause an increase in Oatp2 expression in rat (Dietrich et al., 2007) and mice (Chen et al., 2007) liver. However, because the magnitude of malnutrition of the rats involved in this study is low and because the effects observed in extreme conditions (such as severe malnutrition or fasting) are contrary to the effects of CRF observed in the present study, we do not believe that the weight difference between our two groups could have influenced our results.

In conclusion, this study shows that CRF is associated with important modifications in the expression and activity of the different liver drug transporters. It appears that uremic toxins or proinflammatory cytokines accumulated in CRF could be responsible for these modifications, which seem to be affecting canalicular and basolateral transporters in a different manner.

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