

# Downregulation of Hepatic Cytochrome P450 in Chronic Renal Failure

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**Abstract.** Chronic renal failure (CRF) is associated with a decrease in drug metabolism. The mechanism remains poorly understood. The present study investigated the repercussions of CRF on liver cytochrome P450 (CYP450). Three groups of rats were defined: control, control paired-fed, and CRF. Total CYP450 activity, protein expression of several CYP450 isoforms as well as their mRNA, and the *in vitro* N-demethylation of erythromycin were assessed in liver microsomes. The regulation of liver CYP450 by dexamethasone and phenobarbital was assessed in CRF rats. Compared with control and control paired-fed rats, creatinine clearance was reduced by 60% ( $P < 0.01$ ) in CRF rats. Weight was reduced by 30% ( $P < 0.01$ ) in control paired-fed and CRF rats, compared with control animals. There was no difference in the CYP450 parameters between control and control paired-fed. Compared with control

paired-fed rats, total CYP450 was reduced by 47% ( $P < 0.001$ ) in CRF rats. Protein expression of CYP2C11, CYP3A1, and CYP3A2 were considerably reduced ( $>40\%$ ,  $P < 0.001$ ) in rats with CRF. The levels of CYP1A2, CYP2C6, CYP2D, and CYP2E1 were the same in the three groups. Northern blot analysis revealed a marked downregulation in gene expression of CYP2C11, 3A1, and 3A2 in CRF rats. Although liver CYP450 was reduced in CRF, its induction by dexamethasone and phenobarbital was present. N-demethylation of erythromycin was decreased by 50% in CRF rats compared with control ( $P < 0.001$ ). In conclusion, CRF in rats is associated with a decrease in liver cytochrome P450 activity (mainly in CYP2C11, CYP3A1, and 3A2), secondary to reduced gene expression.

Reduction in renal function alters the disposition of many drugs mainly by decreasing the elimination of those excreted by the kidney (1,2). However, drug metabolism by the liver may also be altered in patients with chronic renal failure (CRF) (3). Indeed, several studies have shown that the metabolic clearance of various substrates is reduced in patients with CRF (1,3,4). The severity of the inhibition of drug metabolism is variable (from 17 to 85%), depending on the metabolic pathway involved, but drugs metabolized by hepatic cytochrome P450 seem particularly vulnerable (3,5). Supporting the hypothesis that CRF inhibits liver P450 is the reduction in hepatic P450 as well as other cytosolic enzymes in rats with experimental renal failure (6–9).

Rat hepatic cytochrome P450 is composed of several isoforms. Those involved in drug metabolism processes include CYP1A2, CYP2C11, CYP2D, CYP2E1, and CYP3A1/3A2 (10). The knowledge of which isoforms are reduced by CRF is critical to predict which drugs are at risk for accumulation. Although previous work has focused on the repercussions of

CRF on total hepatic P450 content, only one has studied whether the reduction in P450 involves all isoforms. Uchida *et al.* (11) demonstrated that in CRF rats, protein expression of some liver P450 isoforms were decreased (CYP2C6, 2C11, and 3A2) while another was increased (CYP1A1). However, the relation between reduced protein expression in P450 isoforms and their metabolic activities has not been studied. Furthermore, the mechanism of liver P450 reduction remains poorly understood; it may be secondary to a decrease in synthesis or an increase in degradation.

The objectives of this study were to determine the effects of CRF on hepatic P450 and to define the mechanisms leading to its downregulation. For this purpose, in control, control paired-fed, and CRF rats, we measured the following: (1) liver cytochrome P450 total activity; (2) the main P450 isoforms involved in drug metabolism, *e.g.*, CYP1A1/1A2, 2C6, 2C11, 2D1, 2E1, 3A1, and 3A2, as well as some of their specific metabolic activities; and (3) the mRNA encoding for these specific isoforms. Finally, the effect of CRF on liver cytochrome P450 was also studied in rats treated with known inducers of P450, dexamethasone, and phenobarbital (12).

## Materials and Methods

### Experimental Model

Male Sprague-Dawley rats (Charles River, Saint-Charles, Québec, Canada), weighing 200 to 300 g, were housed in the Research Center animal care facility and maintained on Purina rat pellets (Ralston-Purina, St. Louis, MO) and water *ad libitum*. The animals were

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allowed an acclimatization period of at least 3 d before any experimental work was undertaken. All of the experiments were conducted according to the Canadian Council on Animal Care guidelines for care and use of laboratory animals.

### Experimental Protocol

Studies were performed in three groups of 10 animals each: control, control paired-fed, and CRF. To evaluate further the effects of CRF on the regulation of liver P450, three other groups ( $n = 6$  in each group) were studied: CRF, CRF treated with dexamethasone, or CRF treated with phenobarbital. Dexamethasone and phenobarbital are potent inducers of liver P450 (12).

Chronic renal failure was induced by a two-staged, five-sixths nephrectomy. Briefly, the rats underwent a two-thirds nephrectomy of the left kidney through a midline incision, and 7 d later, the right nephrectomy was done. Rats from both control groups underwent two sham laparotomies. Pentobarbital was used for anesthesia (60 mg/kg via intraperitoneal injection). After surgery, CRF animals were fed Purina rat chow and water *ad libitum*. Control rats were fed *ad libitum*. Control paired-fed rats were fed the same amount of rat chow that was ingested by the CRF rats on the previous day to assess the effect of CRF-induced malnutrition. Body weight was measured every other day for the duration of the study. 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 killed by decapitation at 42 d after nephrectomy. Blood was collected for the measurement of serum creatinine and urea. Enzyme induction of liver P450 was achieved using an intraperitoneal injection of dexamethasone (100 mg/kg per d) or phenobarbital (80 mg/kg) on days 38 to 41 (12).

### Preparation of Liver Microsomes

The rat livers were immediately excised after death, and microsomes were isolated by differential centrifugation (13). Samples were maintained at 4°C during microsome preparation. Briefly, 5 g of liver was homogenized, using a Potter-Elvehjem tissue grinder (Wheaton Science Products, Millville, NJ), in 25 ml of 0.25 M sucrose and was centrifuged at  $12,000 \times g$ . To the  $12,000 \times g$  supernatant, 1 M  $\text{CaCl}_2$  was added (10% vol/vol) and further centrifuged at  $27,000 \times g$ . The pellet containing the microsomes was stored at  $-80^\circ\text{C}$  in Tris 0.1 M (pH 7.4), glycerol 20%, ethylenediaminetetraacetate (EDTA) 10 mM up to analysis.

### Determination of Total Cytochrome P450 Activity

Microsomal protein content was determined by the method of Lowry *et al.* (14), using bovine serum albumin as standard protein. Total cytochrome P450 activity was measured from the difference spectrum of the reduced protein according to a previously published method (15).

### Western Blot Analysis

The major cytochrome P450 isoforms implicated in the metabolism of drugs were assessed by Western blot analysis: CYP1A2, CYP2C6, CYP2C11, CYP2D, CYP2E1, CYP3A1, and CYP3A2. Forty  $\mu\text{g}$  of protein was electrophoresed in a 7.5% polyacrylamide gel containing 0.1% sodium dodecyl sulfate (SDS), and separated proteins were electrophoretically transferred on nitrocellulose (16,17). Immunoblots for respective isoforms were performed in 5% low-fat milk in phosphate-buffered saline and washed with 0.1% Tween 20 in phosphate-buffered saline. CYP1A2 was detected using a polyclonal goat anti-rat 1A2 (Gentest Corporation, Woburn, MA). CYP2C6 and CYP2C11

were detected using a goat anti-rat 2C6 and 2C11 (Gentest), respectively. CYP2D was detected using a rabbit anti-human 2D (Oxford Biochemical Research Inc., Oxford, MI). CYP2E1 and CYP3A1 were detected using a monoclonal mouse anti-rat 2E1 and 3A1 (Oxford), respectively. CYP3A2 was detected using a goat anti-rat 3A2 (Gentest). Immune complexes were revealed by secondary antibody (swine anti-goat IgG and goat anti-rabbit IgG [Biosource International, Cammarillo, CA], as well as goat anti-mouse IgG [Sigma Chemicals, St. Louis, MO]) coupled to peroxidase and the Luminol derivative of Lumi-Light Western blotting substrate (Roche Diagnostics, Laval, Québec, Canada). Immune reaction intensity was determined by computer-assisted densitometry on exposed Biomax MR film (Scientific Imaging Systems, Eastman Kodak Co., Rochester, NY).

### mRNA Analysis

At the time of death, biopsies of liver were rinsed in ice-cold saline and flash-frozen in liquid nitrogen. Samples were kept at  $-80^\circ\text{C}$  until RNA extraction. The RNA encoding for CYP1A2, CYP2C11, CYP3A1, and CYP3A2 was evaluated by Northern blot analysis. Total RNA was extracted from frozen tissue by the RNeasy kit (Qiagen, Mississauga, Ontario, Canada). RNA concentrations were determined by measuring absorbance at a wavelength of 260 nm.

Total RNA samples were denatured by heating at  $65^\circ\text{C}$  in buffer containing 42% deionized formamide, 30 mM 4-morpholinepropane-sulfonic acid, and 8.5% formaldehyde. RNAs (30  $\mu\text{g}$  total RNA) were separated by electrophoresis in 1% agarose-1.7% formaldehyde gel submerged in buffer (pH 7.2), containing 20 mM 4-morpholinepropane-sulfonic acid, 8 mM sodium acetate, and 1 mM EDTA. Separate RNA were transferred to nylon membranes (Qiabran, Qiagen), using the standard capillary technique with  $10 \times \text{SSC}$  (1.5 M NaCl and 0.15 M sodium citrate, pH 7.0) and fixed under ultraviolet lamp at 0.6 J/cm<sup>2</sup>. Prehybridizations were performed at  $52^\circ\text{C}$  in buffer composed of 0.5 M  $\text{NaPO}_4$  (pH 7.2), 7% SDS, 1% bovine serum albumin, 1% dextran sulfate, 1 mM EDTA, and 250  $\mu\text{g}/\text{ml}$  denatured herring sperm DNA (Roche Diagnostics). The blots were hybridized with oligonucleotide probes specific for each P450 mRNA: for CYP1A2, a synthetic 20-mer oligonucleotide complementary to bases 1580 to 1599 of the P450 1A2 cDNA sequence (18); for CYP2C11, a 30-mer oligonucleotide corresponding to the complement of nucleotides 945 to 974 of the coding sequences of P450 2C11 (19); for CYP3A1, a synthetic 32-mer oligonucleotide complementary to bases 1593 to 1624 of the P450PCN1 nucleotide sequence (20); for CYP3A2, a synthetic 24-mer oligonucleotide complementary to bases 1652 to 1675 of the 6 $\beta$ -A nucleotide sequence (21); for 18S ribosomal RNA, a 1.5-kb human cDNA insert from *EcoRI* site of the pBluescript SK-vector ATCC no.77242 (MBI Fermentas). Oligonucleotides were prepared by the Sheldon Biotechnology Center (McGill University, Montreal, Québec, Canada). They were end labeled with [ $\gamma$ -<sup>32</sup>P]ATP (3000 Ci/mmol), using T4 polynucleotide kinase (BRL, Burlington, Ontario, Canada). The 18S cDNA probe was labeled using [ $\alpha$ -<sup>32</sup>P]dCTP (3000 Ci/mmol) and Klenow according to the random oligo-priming method. Hybridization was then performed at  $52^\circ\text{C}$  for 24 h in prehybridization buffer to which were added the labeled probes. The membranes were then washed in 0.2 M  $\text{NaPO}_4$  (pH 7.2), 1% SDS, 1 mM EDTA at room temperature and at  $52^\circ\text{C}$ . Washed membranes were exposed to autoradiography film (Biomax MS; Kodak) with Biomax TranScreen-HE intensifying screen (Kodak) at  $-80^\circ\text{C}$  for 3 to 10 d. Hybridization signals were quantified by computer-assisted densitometer. mRNA levels were expressed as arbitrary densitometric units and standardized by comparison with hybridization results obtained with 18S ribosomal RNA probe.

### *In Vitro Metabolism of Erythromycin*

To evaluate the metabolic activity of CYP3A2 in liver microsomes, erythromycin N-demethylation was determined as described by Wang *et al.* (22). Erythromycin (250  $\mu$ M) (Sigma Chemicals) was incubated with 2.0 mg of rat liver microsomes (either from control paired-fed or CRF) at 37°C for 15 min in the presence of an NADPH-generating system consisting of the following: 10 mM glucose 6-phosphate, 1 mM NADP, and 0.35 units glucose-6-phosphate dehydrogenase (Sigma Chemicals) in a total volume of 0.5 ml. Reactions were quenched with 0.05 ml of 25% ZnSO<sub>4</sub> and 0.05 ml of 0.3N Ba(OH)<sub>2</sub>. The samples were then centrifuged at 14,000  $\times$  g for 10 min, and 0.35 ml of the supernatant was transferred and mixed with 0.15 ml of Nash reagent (23). The mixture was incubated at 56°C for 30 min, and samples were analyzed by spectrophotometry (absorbance 405 nm) to determine the formation of formaldehyde (23).

### *Other Assays*

Blood and urine chemistries were determined with a Hitachi 717 autoanalyser (Boehringer Mannheim Canada, Laval, Québec, Canada).

### *Statistical Analyses*

The results are expressed as mean  $\pm$  SEM. Differences between groups were assessed by using an unpaired *t* test or an ANOVA test. Significant ANOVA was followed by Fisher least significant difference multiple comparisons procedure. The threshold of significance was  $P < 0.05$ .

## **Results**

### *Biochemical Parameters and Body Weight in Control, Control Paired-Fed, and CRF Rats*

Table 1 presents the biochemicals and body weight of the three groups of animals studied. Compared with control and control paired-fed animals, CRF rats had higher levels of plasma creatinine and urea and lower values of creatinine clearance (reduced by 60%;  $P < 0.001$ ). Body weight in control paired-fed and CRF rats was reduced by 30% compared with control animals. However, there was no difference in body weight between control paired-fed and CRF rats.

### *Liver Total Cytochrome P450 Activity in Control, Control Paired-Fed, and CRF Rats*

No difference was observed in total cytochrome P450 activity between control and control paired rats ( $0.61 \pm 0.01$  and  $0.59 \pm 0.04$  nmol/mg protein; Table 1). Thus, malnutrition as

produced by CRF has no effect on liver P450. In CRF rats, total cytochrome P450 activity was significantly reduced by 47% compared with both control groups (Table 1). The P450 activity was negatively correlated with creatinine clearance ( $r = 0.68$ ,  $P < 0.001$ ). Similar correlations were found between P450 activity and blood urea and creatinine.

### *Protein Expression of Liver Cytochrome P450 Isoforms in Control, Control Paired-Fed, and CRF Rats*

No differences in the different isoforms between control and control paired-fed rats were observed (data not shown). The levels of CYP2C11, 3A1, and 3A2 in CRF rats were reduced by 40, 75, and 65%, respectively, in CRF rats compared with control paired-fed animals ( $P < 0.001$ ; Figure 1). Conversely, the levels of CYP1A2, 2C6, 2D1, and 2E1 were not modified in CRF rats compared with control rats.

### *mRNA Encoding Liver Cytochrome P450 Isoforms in Control, Control Paired-Fed, and CRF Rats*

To determine whether liver cytochrome P450 isoforms in CRF were downregulated secondary to a decrease in their synthesis or an increase in their degradation, we evaluated mRNA encoding the different isoforms by Northern blot analysis. Again, there was no difference in the level of mRNA between control and control paired-fed rats (data not shown). However, a significant decrease in mRNA encoding CYP2C11, 3A1, and 3A2 isoforms was observed in CRF compared with control paired-fed animals (Figure 2). Thus, the decrease in protein expression of the different isoforms of P450 observed in CRF is secondary to reduced gene expression.

### *In Vitro Metabolism of Erythromycin in Control, Control Paired-Fed, and CRF Rats*

To determine the repercussion of cytochrome P450 reduction in CRF on the metabolism of drugs, we assessed the *in vitro* N-demethylation of erythromycin in liver. This enzymatic reaction is mediated primarily by the CYP3A family. No differences between control and control paired-fed rats were observed (data not shown). The N-demethylation of erythromycin was decreased by more than 50% in rats with CRF, compared with control paired-fed animals ( $P < 0.001$ ; Figure 3).

Table 1. Characteristics of the control, control paired-fed and CRF rats<sup>a</sup>

	Control	Control Paired-Fed	CRF
Body weight (g)	459 $\pm$ 3.7	318 $\pm$ 9.4 <sup>b</sup>	327 $\pm$ 8.5 <sup>b</sup>
Serum creatinine ( $\mu$ mol/L)	52 $\pm$ 1	56 $\pm$ 2	141 $\pm$ 9 <sup>b,c</sup>
Creatinine clearance ( $\mu$ l/100 g of body weight/min)	406 $\pm$ 18	390 $\pm$ 17	178 $\pm$ 25 <sup>b,c</sup>
Serum urea (mmol/L)	4.8 $\pm$ 0.2	5.9 $\pm$ 0.5	24.5 $\pm$ 5.8 <sup>b,c</sup>
Liver total cytochrome P450 activity (nmol/mg of proteins)	0.61 $\pm$ 0.01	0.59 $\pm$ 0.04	0.34 $\pm$ 0.04 <sup>b,c</sup>

<sup>a</sup> CRF, chronic renal failure. Data are the mean  $\pm$  SEM. Measurements were made 41 d after the first surgery.

<sup>b</sup>  $P < 0.01$  compared with control rats.

<sup>c</sup>  $P < 0.01$  compared with control paired-fed animals.

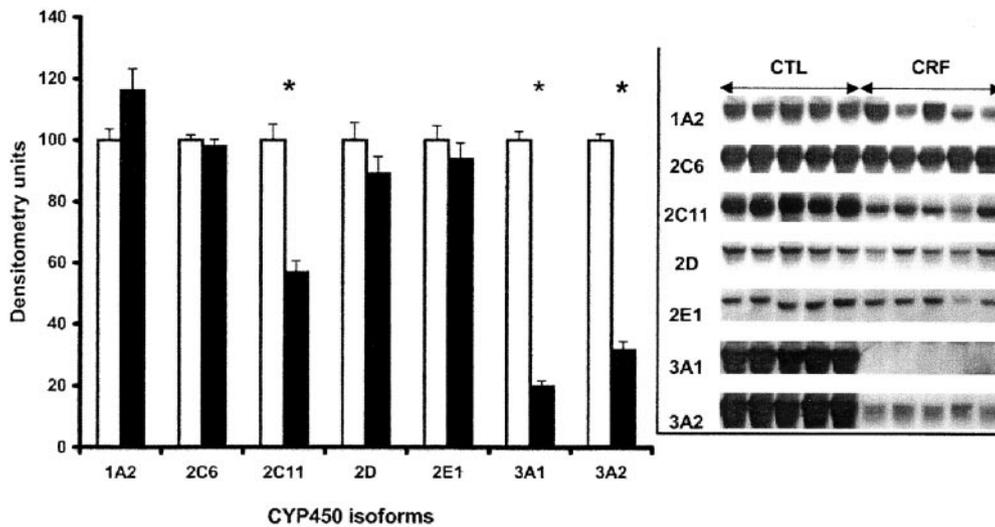


Figure 1. Protein expression of liver cytochrome P450 isoforms in control paired-fed (□) and chronic renal failure (CRF) rats (■). Protein bands are expressed in densitometry units (%). The densitometry units of control paired-fed rats were arbitrarily defined as 100%. Data are the mean ± SEM of 10 rats in each group. \*,  $P < 0.001$  as compared with control paired-fed rats. Representative blots are also shown.

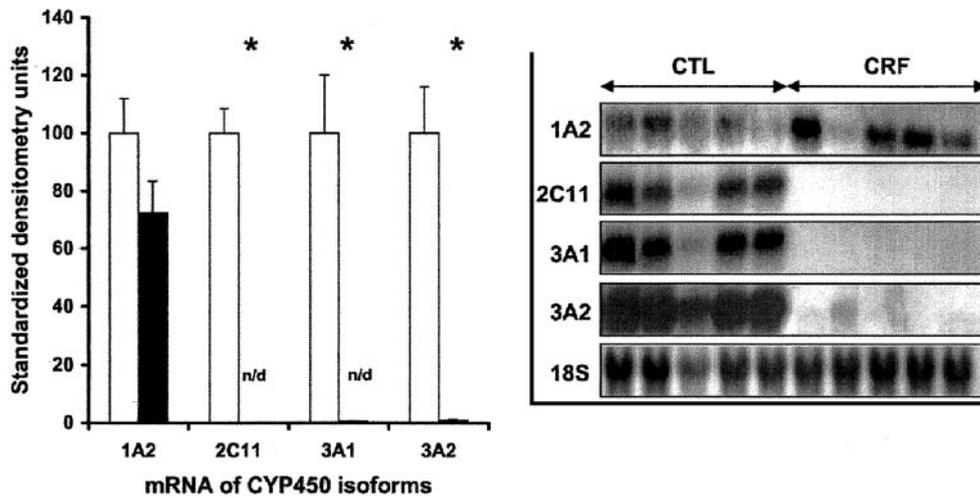


Figure 2. mRNA encoding liver cytochrome P450 isoforms (CYP1A2, CYP2C11, CYP3A1, and 3A2) in control paired-fed (□) and CRF rats (■). mRNA bands are expressed in standardized densitometry units (%). The densitometry values for cytochrome P450 isoforms were standardized by dividing these values by the values for 18S ribosomal RNA. The standardized densitometry units of control paired-fed rats were arbitrarily defined as 100%. Data are the mean ± SEM of six rats in each group. \*,  $P < 0.01$  as compared with control paired-fed rats. Representative blots are also shown.

*Induction of Cytochrome P450 in Rats with CRF by Dexamethasone or Phenobarbital*

Although our results showed that the decrease in liver cytochrome P450 in CRF is secondary to reduced gene expression, we were also interested to know whether P450 was still inducible, despite its inhibition by CRF. We studied the effect of dexamethasone and phenobarbital (which are potent inducers of the CYP3A family) in CRF rats on CYP3A1 and 3A2 protein expressions as well as on their mRNA levels (Figure 4). In CRF rats, CYP3A1 and 3A2 were greatly enhanced by dexamethasone and phenobarbital. This was secondary to an upregulation of mRNA encoding these proteins. We further studied the effect of dexamethasone on N-demethylation of

erythromycin. In CRF rats, N-demethylation of erythromycin was also increased by dexamethasone ( $0.28 \pm 0.02$  versus  $1.38 \pm 0.07$  nmol/mg of protein/min,  $P < 0.001$ ).

**Discussion**

This study demonstrates that in the rat, CRF induces a marked decrease in liver total cytochrome P450 activity secondary to reduced protein expression of selective cytochrome P450 isoforms, namely CYP2C11, 3A1, and 3A2. The mechanism underlying this downregulation is a reduction in the mRNA levels encoding these proteins. The repercussions on the metabolism of drugs by the liver are important in that we observed a 50% reduction of erythromycin biotransformation

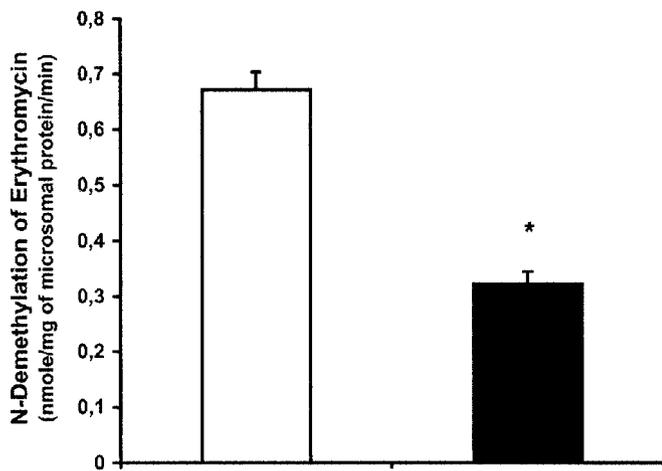


Figure 3. *In vitro* metabolism of erythromycin (N-demethylation) in control paired-fed (□) and CRF rats (■). Incubations were performed with 2 mg of rat liver microsome at 37°C for 15 min. Data are the mean  $\pm$  SEM of six rats in each group. \*,  $P < 0.001$  as compared with control paired-fed rats.

mediated by the CYP3A family. Although liver cytochrome P450 is decreased in CRF, its dexamethasone and phenobarbital regulation was still present as shown by the induction of CYP3A by dexamethasone or phenobarbital.

Renal failure has been generally thought to decrease only the renal clearance of drugs (24). However, several studies have demonstrated that animals with CRF also present decreased hepatic drug metabolism (5). Because the P450 is the major enzymatic system involved in drug metabolism, most studies have focused on liver P450. The results of these studies show that in CRF rats there is a 18.6 to 42.8% decrease in liver total P450 (6–9,11). Furthermore, important reductions in enzymatic reactions normally carried by the liver P450 have been reported: N-demethylation of aminopyrine and ethylmorphine, O-demethylation of codeine, and hydroxylation of aniline (5). In the present study, we found a 47% reduction in total P450 activity as well as a significant reduction in the N-demethylation of erythromycin.

Few studies have focused on the specific P450 isoforms reduced in CRF (11). Knowledge of which isoform is reduced by CRF is critical to predict which drugs are at risk for accumulation when used in CRF. Recently, Uchida *et al.* (11) reported a reduction in the levels of hepatic CYP2C6, CYP2C11, and CYP3A2 and a slight increase in CYP1A2 in rats with CRF. Our results demonstrate that only CYP2C11, CYP3A1, and CYP3A2 are significantly reduced, while no isoform induction was noted. Furthermore, the level of reduction was far more important in the present study. This could reflect a more pronounced degree of uremia obtained in our rats (50% increase in plasma creatinine *versus* 65% in the present study) and also a longer period of uremia (21 d *versus* 42 d in the present study). Interestingly, CYP3A1 and 3A2 in the rat correspond to CYP3A4 in humans. Because this isoform is responsible for the metabolism of several drugs commonly used in CRF patients, patients with CRF could be at risk for drug accumulation and toxicity.

Our results demonstrate that there is an association between lower levels of mRNA and protein for some isoforms of cytochrome P450, namely CYP2C11, 3A1, and 3A2 (Figure 2). This suggests that there is reduced gene expression. The mechanisms responsible for the diminished liver CYP2C11, 3A1, and 3A2 gene expression in CRF are not known. Caloric restriction, as seen in CRF rats, downregulates hepatic genes of drug metabolizing enzymes in the mouse and in the rat (25,26). However, in our control paired-fed rats, we did not observe any modification in cytochrome P450 levels despite a similar weight loss as in CRF rats. Further studies will require evaluation of uremia on the function of the CYP450 promoters.

A number of studies indicate that animals with CRF display impaired protein synthesis, by reduced gene expression, in the liver as well as in the skeletal muscle (27,28). For instance, the mRNA of hepatic lipase and insulin-like growth factor 1 receptor are decreased in hepatocytes and skeletal muscle, respectively, of rats with CRF (27,28). CRF is associated with sustained elevations in calcium in many cell types, including the hepatocytes as well as the skeletal muscle, and this high intracellular calcium seems to be a major factor underlying cell dysfunction and reduced protein synthesis in CRF (29,30). However, the effects of intracellular calcium on the regulation of P450 remain poorly defined (31,32). Whether increased intracellular calcium is implicated in the downregulation of liver P450 in CRF remains to be studied.

In the present study, downregulation of cytochrome P450 in CRF was overcome by dexamethasone and also by phenobarbital, which are potent inducers of CYP3A isoforms (Figure 4). This result suggests that although liver cytochrome P450 is decreased in CRF, its dexamethasone and phenobarbital regulation is still present. The clinical significance of this finding remains to be defined. However, one can anticipate that in CRF, liver cytochrome P450 could be modulated by known P450 inducers, *e.g.*, steroids, phenobarbital.

Several investigators have demonstrated that in patients with renal failure, there is also a decrease in the metabolic clearance of many drugs (ranging from 17 to 85%) (1,3,4). The vast majority of these drugs are metabolized by the liver through the cytochrome P450 pathway. Unfortunately, we are not aware of human studies demonstrating a reduction in liver cytochrome P450 isoforms in CRF. However, several methods have been described to assess *in vivo* the CYP activity in humans, and the most widely used is the administration of probe drugs that are selectively metabolized by a specific CYP isoform. Kevorkian *et al.* (33) reported that assessment of CYP2D6 activity by the use of dextromethorphan or sparteine was possible in patients with CRF. The results of their study revealed that there was a decrease in the metabolic clearance of sparteine suggesting a decrease in CYP2D6 activity in CRF patients. There seems to be a correlation between the decrease in the metabolism of drugs and the severity of renal failure in humans (34). Interestingly, in the rat, we found a significant correlation between the decrease in renal function and the reduction in liver cytochrome P450 and also with the reduction in the N-demethylation of erythromycin. These results suggest that as CRF

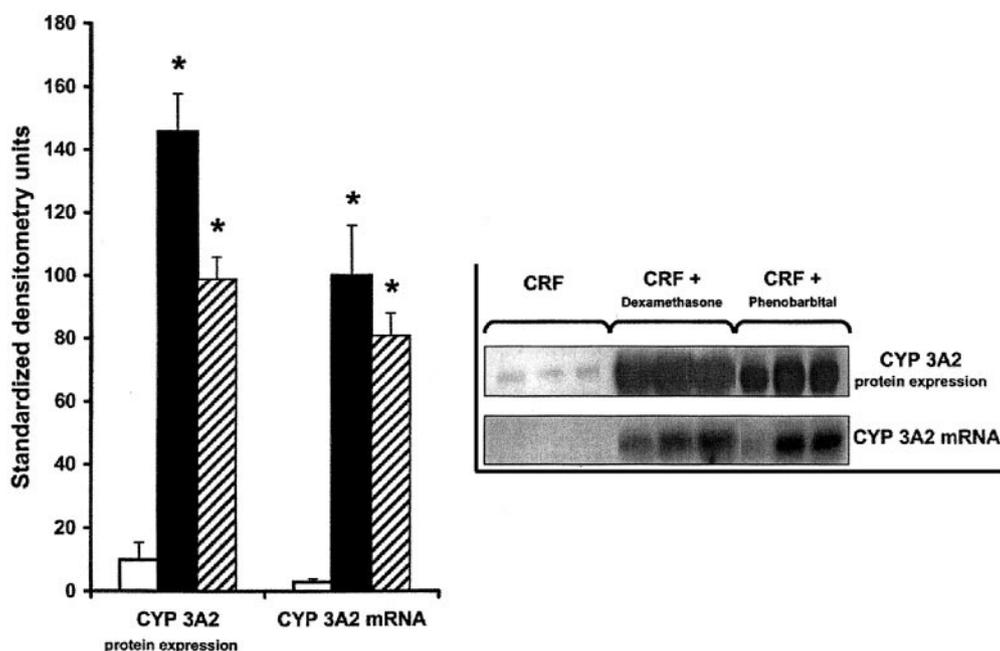


Figure 4. Changes in liver microsome levels of CYP3A2 protein expression and specific mRNA in response to treatment with dexamethasone (100 mg/kg intraperitoneally daily during 4 d) (■) or with phenobarbital (80 mg/kg intraperitoneally daily during 4 d; &U25A8;). Data are the mean  $\pm$  SEM of six rats in each group. \*,  $P < 0.001$  as compared with CRF without inducer treatment (□). Representative blots are also shown.

worsened, patients were at risk of drug accumulation and toxicity, secondary to reduction in their metabolism.

In conclusion, CRF is associated with a decrease in liver cytochrome P450 isoforms in rats (mainly CYP2C11, CYP3A1, and 3A2), secondary to reduced mRNA levels. Drug metabolism activity, assessed by the N-demethylation of erythromycin, is also greatly depressed in CRF rats. Liver cytochrome P450 downregulation is correlated with the degree of renal failure. This decrease could explain the reduction in drug metabolism observed in CRF patients because these isoforms (especially CYP3A1 and 3A2) correspond to CYP3A4 in humans, which is responsible for the metabolism of several drugs commonly used in CRF patients.

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