1521-009X/44/8/1174–1179$25.00
Drug Metabolism and Disposition

http://dx.doi.org/10.1124/dmd.116.070052
Drug Metab Dispos 44:1174–1179, August 2016

Effects of Chronic Renal Failure on Brain Cytochrome P450 in Rats[S]

Judith Naud, Jessica Harding, Caroline Lamarche, Stephanie Beauchemin, Francois A. Leblond, and Vincent Pichette

Service de néphrologie et Centre de recherche de l'Hôpital Maisonneuve-Rosemont (J.N., J.H., C.L., S.B., F.A.L., V.P.), and Département de pharmacologie (J.N., J.H., V.P.), Faculté de Médecine, Université de Montréal, Québec, Canada

Received February 12, 2016; accepted May 18, 2016

ABSTRACT

Chronic renal failure (CRF) impedes renal excretion of drugs and their metabolism by reducing the expression of liver cytochrome P450 (P450). Uremic serum contains factors, such as parathyroid hormone (PTH), that decrease liver P450s. The P450s are also involved in the metabolism of xenobiotics in the brain. This study investigates: 1) the effects of CRF on rat brain P450, 2) the role of PTH in the downregulation of brain P450s in CRF rats, and 3) the effects of PTH on P450s in astrocytes. Protein and mRNA expression of P450s were assessed in the brain of CRF and control (CTL) rats, as well as from CTL or CRF rats that underwent parathyroidectomy (PTX) 1 week before nephrectomy. CYP3A activity was measured using 3-[(3, 4-difluorobenzyl) oxy]-5, 5-dimethyl-4-[4-methylsulfonyl) phenyl] furan-2(5H)-1 metabolism in brain microsomal preparation. CYP3A protein expression was assessed in primary cultured astrocytes incubated with serum obtained from CRF or CTL rats or with PTH. Significant downregulations (≥40%) of CYP1A, CYP2C11, and CYP3A proteins were observed in microsomes from CRF rat brains. CYP3A activity reduction was also observed. CYP3A expression and activity were unaffected in PTX-pretreated CRF rats. Serum of PTX-treated CRF rats had no impact on CYP3A levels in astrocytes compared with that of untreated CRF rats. Finally, PTH addition to normal calf serum induced a reduction in CYP3A protein similar to CRF serum, suggesting that CRF-induced hyperparathyroidism is associated with a significant decrease in P450 drug-metabolizing enzymes in the brain, which may have implications in drug response.

Introduction

Cytochrome P450 (P450) proteins are the major catalyst of phase 1 metabolism. They are involved in the oxidative metabolism of endogenous and exogenous substrates such as xenobiotics, cholesterol, steroids, and vitamin D metabolism. P450 expression is highest in the liver but can also be found in various organs, including the intestine, kidneys, lungs, and brain.

Brain P450s are involved in the metabolism of xenobiotics, such as antidepressants, opiates, steroids, and antipsychotics, as well as endobiotics, such as steroids, arachidonic acid, dopamine, and serotonin (Miksys and Tyndale, 2013). P450 isoforms such as CYP1A1/2, CYP2B1, CYP2C11, CYP2D1–6, CYP2E1, CYP3A1, and CYP4F have been identified in rat brain (Morse et al., 1998; Imaoka et al., 2005; Yadav et al., 2006; Miksys and Tyndale, 2013). Although the overall expression of individual P450s in the brain is low, high activity of specific P450 enzymes can be found in different brain regions, such as the hippocampus, cerebellum, cortex, and hypothalamus, where these proteins are suggested to participate in local endobiotic and xenobiotic metabolism (Miksys and Tyndale, 2002; Yadav et al., 2006; Meyer et al., 2007).

Over the past 15 years, many studies have demonstrated that chronic renal failure (CRF) significantly affects the expression and activity of the liver, intestine, and kidney P450, thus altering extrarenal drug metabolism (Leblond et al., 2000, 2001, 2002; Naud et al., 2011, 2012b) and vitamin D metabolism (Michaud et al., 2010). Parathyroid hormone (PTH) has been identified as a major factor responsible for P450 downregulation in CRF rats (Michaud et al., 2006, 2010). In light of these studies, we hypothesized that CRF could also impede brain P450 expression or activity and that PTH could be involved in brain P450 modulation.

The objective of this study was thus to investigate the effect of CRF on P450s in whole or specific regions of the rat brain and the involvement of PTH in it. To address this, we have measured the protein and mRNA expression of P450s in the cerebellum, cortex, hippocampus, and remaining brain tissue from CRF and control (CTL) rats. To assess the involvement of PTH, we measured the protein expression and the activity of CYP3A in microsomes purified from brains of CRF, parathyroidectomized-CRF (CRF-PTX), parathyroidectomized-control (CTL-PTX), and control rats. To investigate further the role of PTH, we measured the expression levels of CYP3A in primary rat astrocytes cultured in the presence of sera obtained from CTL, CRF, CRF-PTX, and CTL-PTX rats. Finally, astrocytes were incubated with a high concentration of PTH to evaluate the effect of PTH on CYP3A expression.

Materials and Methods

Experimental Model. Male Sprague-Dawley rats (Charles River, Portage, MI), weighing 176–225 g were housed in the research center animal care facility and maintained on Harlan Teklad rodent diet (Harlan Teklad Global Diets, Montreal, Canada) and water ad libitum. Animals were allowed an acclimatization period of at least 7 days before the first nephrectomy was performed. All the experiments were conducted according to the Canadian Council on Animal Care

This work was financed by La Fondation de l'Hôpital Maisonneuve-Rosemont (La néphrologie et son Impact). V. Pichette is supported by a career award from Fonds de la recherche en santé du Québec (FRSQ).
dx.doi.org/10.1124/dmd.116.070052.
[S]This article has supplemental material available at dmd.aspetjournals.org.

ABBREVIATIONS: CRF, chronic renal failure; CTL, control; DFB, 3-[(3,4-difluorobenzyl)oxy]-5,5-dimethyl-4-[4-methylsulfonyl)phenyl]furan-2(5H)-one; DFH, 3-hydroxy-5,5-dimethyl-4-[4-(methylsulfonyl)phenyl]furan-2(5H)-one; PBS, phosphate-buffered saline; P450, cytochrome P450; PTH, parathyroid hormone; PTX, parathyroidectomy.

guidelines for the care and use of laboratory animals and under the supervision of our local animal care committee.

CRF was induced by two-stage 5/6 nephrectomy as described previously (Leblond et al., 2001). Rats from the control group also underwent two sham laparotomies (on days 1 and 8). Every animal had ad libitum access to water, but to limit the effects of CRF-induced malnutrition, control pair-fed rats were fed the same amount of chow that CRF rats ate on the previous day. At day 41 after the nephrectomy, the rats were housed in metabolic cages, and urine was collected for 24 hours to determine the clearance of creatinine. Rats were sacrificed by decapitation at 42 days for brain and blood collection. Brain was immediately excised, rinsed in ice-cold saline, and then either dissected fractions (cerebellum, cortex, hippocampus, and remaining brain parenchyma, $n = 12$ CTL and 13 CRF) or whole-brain ($n = 13$ CTL, 8 CTL-PTX, 13 CRF, 11 CRF-PTX) were flash-frozen in liquid nitrogen. Samples were stored at −80°C until microsome preparation or mRNA extraction. After collection, blood was rapidly stored on ice. After coagulation, serum was recovered by centrifugation (600 g for 10 minutes at 4°C), and samples were kept for the measurement of serum creatinine, urea, and PTH, and for bioassays with cultured rat astrocytes. The remaining sera were stored at −80°C.

Total parathyroidectomy (PTX) was performed as previously described (Klin et al., 1996; Michaud et al., 2006, 2010). Briefly, surgical PTX was carried out under a surgical microscope, without removal of the thyroid tissue. The success of the PTX was ascertained by a significant decrease in calcium after PTX. To avoid hypocalcemia, PTX animals were then supplemented with calcium by adding calcium gluconate to drinking water (control 5%; CRF 2.5%). Rats were then allowed to recover for a week before 5/6 nephrectomy. Control rats received sham surgery in the neck region.

Preparation of Brain Microsomes. Microsomes from cerebellum, cortex, hippocampus, and remaining brain parenchyma or from whole brain were isolated as published by Cinti and colleagues (Cinti et al., 1972; Leblond et al., 2000). The resulting pellet was suspended in either phosphate-buffered saline (PBS) containing 0.1 mM phenylmethylsulfonylfluoride for Western blot analysis or in sucrose 0.25 M for CYP3A activity assessment. Samples were then sonicated on ice for 10 seconds to ensure homogeneity. Protein concentration was determined using the method of Lowry (Lowry et al., 1951), using bovine serum albumin as a reference protein. Aliquots were stored at −80°C up to Western blot analysis or CYP3A activity assessment.

Western Blot Analysis. Brain P450s levels were assessed by Western blot analysis according to a previously described protocol (Leblond et al., 2002; Naud et al., 2007, 2008, 2011). Briefly, proteins were separated on 9% SDS-PAGE and transferred to nitrocellulose membrane. Nonspecific proteins were blocked by incubating Western blot membranes in 5% skim milk in PBS. P450 proteins were detected using specific antibodies. CYP1A1/2 and CYP2E1 antibodies were from US Biologicals (Salem, MA), and CYP2C11, CYP2D, and CYP3A antibodies were from Abcam (Cambridge, MA), Creative Biomart (Shirley, NY) and Millipore (Billerica, MA), respectively. β-Actin, used as a loading control, was detected using a mouse anti-chicken β-actin (Neo-Markers, Fremont, CA). Secondary antibodies against mouse or rabbit IgG were from Sigma (St. Louis, MO). Antibodies were diluted in 0.5% skim milk, and membranes were washed with 0.1% Tween 20 in PBS. All blots were revealed using Lumi-light chemiluminescent reagents (Roche Diagnostics, Laval, Canada). Each membrane was probed sequentially for a specific CYP isoform then for β-actin.

Individual P450 protein levels were quantified by measuring the density of bands using ImageQuant LAS 4000 software (GE Healthcare, Mississauga, Canada). The measured density value was reported as a ratio over the expression of β-actin band intensity. Comparison of control and CRF groups was always performed on the same membrane. The mean CYP/β-actin ratio for the control group was arbitrarily defined as 100%, and all samples were expressed as a percentage of this value. Each blot was repeated in two independent experiments.

mRNA Analysis. Total RNA was extracted from frozen tissue using Trizol reagent (Invitrogen, Burlington, Canada) according to the manufacturer's protocol. RNA concentration was determined by measuring absorbance at a wavelength of 260 nm. Total RNA (1 μg) was used to prepare cDNA by reverse transcription using SuperScript VILO cDNA Synthesis Kit from Invitrogen. Gene expressions were measured using appropriate TaqMan gene expression assays from Applied Biosystems (Carlsbad, CA). Table 1 shows the TaqMan gene expression assays used for the quantification of mRNA for each enzyme. Gene expression was determined using the $\Delta\Delta C_T$ method (Livak and Schmittgen, 2001) using β-actin expression as a reference.

TABLE 1

TaqMan gene expression assays used for real-time polymerase chain reaction

Gene	TaqMan Gene Expression Assay
Actb	Rn00667869_m1
CYP1A1	Rn00487218_m1
CYP2C11	Rn00569868_m1
CYP2D1	Rn01775090_mH
CYP2E1	Rn01759587_m1
CYP3A1	Rn03062228_m1

Evaluation of CYP3A Activity. To evaluate the metabolic activity of CYP3A in 200 μg of microsomes prepared from whole-brain extract of CTL, CTL-PTX, CRF, and CRF-PTX rats, a selective fluorescent probe, 3-[(3,4-difluorobenzyl)-oxy]-5,5-dimethyl-4-[4-methylsulfonyl)phenyl]furan-2(5H)-one (DFB), specifically metabolized by rat CYP3A to 3-hydroxy-5, 5-dimethyl-4-[4-(methylsulfonyl) phenyl] furan-2(5H)-one (DFH) was used as previously reported (Nicoll-Griffith et al., 2004; Michaud et al., 2007, 2008). DFH fluorescence was determined on a cytofluorometer (Cytofluor 4000/TR; Perspective Biosystems, Framingham, MA) using appropriate wavelength (excitation filter: 360/40 nm; emission filter: 460/40 nm). The standard curve was prepared with known dilutions of DFH.

Isolation and Culture of Rat Astrocytes. To assess the impact of uremic serum or PTH on the ex vivo expression of P450 in the brain, we incubated rat astrocytes with serum from nephrectomized and PTX rats or with PTH and evaluated CYP3A protein expression. Rat astrocytes were isolated and cultured as previously described from unknown gender newborn rat pups (Booher and Sensenbrenner, 1972; Perriere et al., 2005; Naud et al., 2012a). When cells reached confluence, the standard culture medium was replaced with culture medium containing 10% serum from CTL, CTL-PTX, CRF, or CRF-PTX rats (one serum/flask) or medium containing 10% calf serum supplemented with 10 nM rat PTH (rat synthetic 1-34 PTH, Sigma) or not. After 48 hours of culture, cells were trypsinized, pelleted, and homogenized in PBS containing 0.1 mM phenylmethylsulfonylfluoride. Protein concentrations were determined using the Micro BCA Protein Assay Kit from Thermo Fisher Scientific (Rockford, IL). For Western blot analysis of CYP3A, 100 μg of protein lysate was used for Western blot analysis of CYP3A.

Other Assays. Blood and urine chemistries were determined with an Architect C1600 clinical analyzer (Abbott, Saint-Laurent, QC, Canada). PTH serum levels were measured using the Rat intact PTH ELISA Kit (Alpco Diagnostics, Salem, NH), which measures intact 1-84 PTH. The lowest detectable level was 29 pg/ml.

Statistical Analysis. Results are expressed as mean ± S.D. Statistical analysis was performed using SPSS software. Differences between groups were assessed using an unpaired Student's t test or an analysis of variance test. Significant analysis of variance was followed by a Scheffe post hoc comparison of groups. The threshold of significance was $P < 0.05$.

Results

Biochemical Parameters in Control and CRF Rats. Table 2 presents the biochemical parameters and body weight of control and CRF rats. As shown in the table, compared with control animals, CRF

TABLE 2

Characteristics of control (CRF) and chronic renal failure (CRF) rats

Group		CTL	CRF
Rats (n)		12	13
Body weight	g	362.8 ± 25.7	371.5 ± 75.9
Serum creatinine	μmol/liter	59.4 ± 3.1	197.9 ± 85.9[a]
Creatinine clearance[b]	μl/100 g per min	244.0 ± 89.9	65.5 ± 27.3[a]
Parathyroid hormone	pg/ml	121.7 ± 26.3	1526.8 ± 568.9[a]

[a] $P < 0.05$ versus control group.

[b] Data are the mean ± S.D. Measurements were made at the time of sacrifice. Twenty-four-hour urinary collection was begun the day before. Blood creatinine, and urine creatinine were determined with an Architect C1600 clinical analyzer (Abbott, Saint-Laurent, Canada).

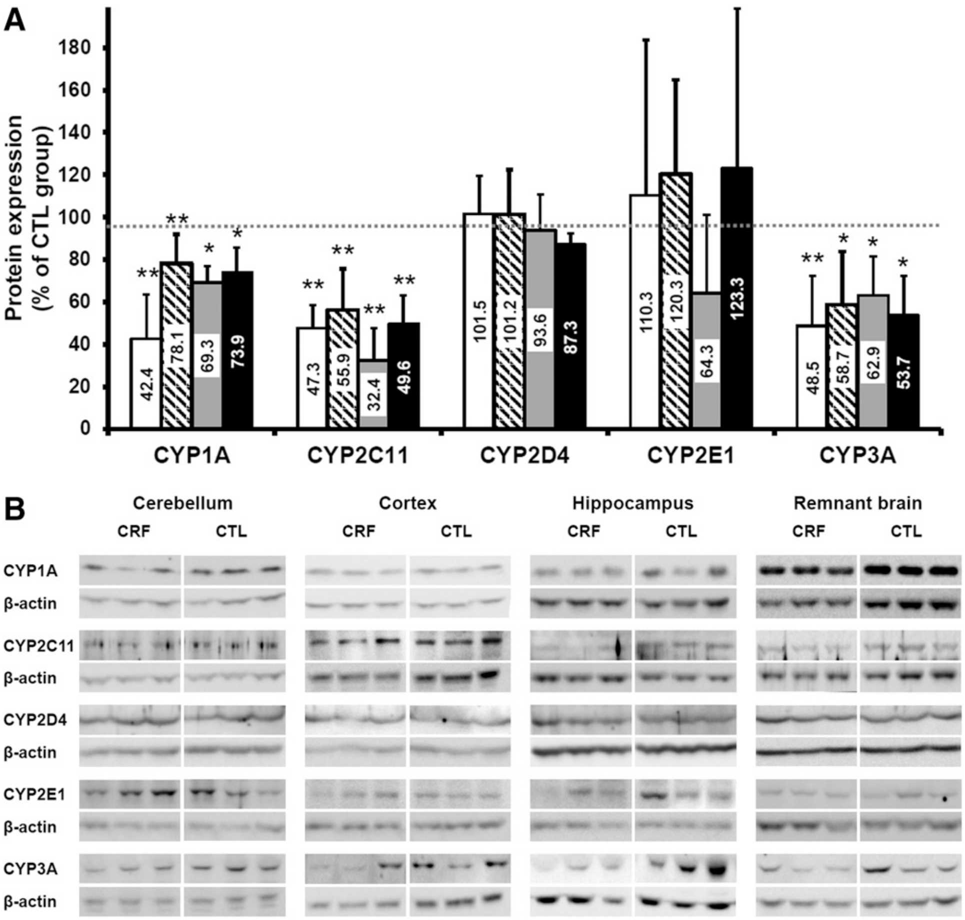

Fig. 1. Protein expression of various P450s in the dissected brain of CRF rats. (A) Protein bands are expressed in densitometry units. The densitometry units measured for P450s in CTL (n = 12) and CRF (n = 13) rats were standardized by dividing them by the value obtained for β-actin. The standardized densitometry units of control rats were arbitrarily defined as 100% and are represented by the dotted line. Graphic shows the mean expression in CRF rats expressed as percent of controls ± S.D. in the cerebellum, cortex, hippocampus, and remaining brain tissue. *$P < 0.05$; **$P < 0.01$ compared with control rats. (B) Typical blot pictures are shown for each P450 isoform and the corresponding β-actin in each part of the brain. For each sub-group (CYP450 / brain part), pictures presented for both CTL and CRF samples were issued from the same blot.

rats had higher levels of serum creatinine and lower creatinine clearance (reduced by 75%, $P < 0.001$). Also, compared with CTL animals, CRF rats had 10-fold higher levels of PTH ($P < 0.05$). There was no difference in body weight between control and CRF rats.

P450 Expression in the Dissected Brain of Control and CRF Rats. First, we tested in control and CRF rats whether CRF could modify the brain P450 expression. Figure 1 shows the protein expression levels of various P450s in cerebellum, cortex, hippocampus, and remaining brain tissue of control and CRF rats. Western blot analysis showed a 25% to 50% ($P < 0.05$) decrease in CYP1A, CYP2C11, and CYP3A protein levels in brain microsomes of CRF rats compared with control group, whereas CYP2D4 and CYP2E1 expression was found similar in both groups. Subdivision of the brain in four parts (cerebellum, cortex, hippocampus, and remaining of the brain) showed that the effect of CRF on the expression of a specific P450 isoform was evenly distributed across the entire organ. Since we found no difference between various parts of the brain, the whole organ was used for subsequent analysis of the effects of PTH on CYP3A expression and activity. When we assessed the mRNA levels of individual P450 isoform in the brain of CRF rats, we did not find any significant modulation between groups (Supplemental Fig. 1).

CYP3A Protein Expression in Whole-Brain Microsomes of CRF and Parathyroidectomized CRF Rats. To evaluate the role of PTH in the in vivo modulation of CYP3A, we surgically removed the parathyroid glands 7 days before the first nephrectomy, thus preventing secondary hyperparathyroidism. Table 3 presents the body weight and

TABLE 3

Characteristics of parathyroidectomized (PTX), control (CTL), and chronic renal failure (CRF) rats

	Nephrectomy	(-)		(+)	
	PTX	(-)	(+)	(-)	(+)
Group		CTL	CTL-PTX	CRF	CRF-PTX
Rats (n)		13	8	13	11
Body weight	g	381.2 ± 20.9	432.0 ± 30.5[a]	317.9 ± 60.4[a]	392.0 ± 43.0
Serum creatinine	μmol/liter	63.3 ± 5.0	66.3 ± 2.4	230.5 ± 74.9[a]	186.4 ± 61.1[a]
Creatinine clearance[b]	μl/100 g per min	288.4 ± 52.9	239.4 ± 36.1	56.6 ± 38.1[a]	76.0 ± 26.8[a]
PTH	pg/ml	113.6 ± 36.8	<29[a]	1304.1 ± 914.7[a]	<29[a]

[a]$P < 0.05$ versus control group.

[b]Data are the mean ± S.D. Measurements were made at the time of sacrifice. Twenty-four hour urinary collection was begun the previous day. Blood creatinine, and urine creatinine were determined with an Architect C1600 clinical analyzer (Abbott, Saint-Laurent, Canada).

biochemical characteristics of parathyroidectomized rats. Compared with control animals, CRF rats had significantly higher levels of PTH ($P < 0.05$), whereas in PTX animals, PTH levels were below the lowest detectable concentration and were unaffected by CRF.

CYP3A protein expression was measured in whole-brain microsomes purified from CTL, CRF, CTL-PTX, and CRF-PTX rats. As shown in Fig. 2, there was a significant decrease in CYP3A protein expression in whole-brain microsomes isolated from CRF rats compared with CTL, and PTX treatment prevented the CYP3A protein level reduction in CRF rats, suggesting that PTX prevents the CYP3A downregulation induced by CRF. No significant modulation of mRNA expression was observed in all groups (Supplemental Fig. 2).

CYP3A Activity in Whole-Brain Microsomes of CRF Rats. To evaluate whether CRF affected CYP3A activity as well, we assessed in vitro, using fluorescently labeled DFB, the enzymatic activity of CYP3A. DFB is converted into DFH by CYP3A, and the fluorescence intensity of DFH is readily measured on a cytofluorometer. To this end, we measured the conversion of DFB into DFH by CYP3A in brain microsomes isolated from CTL, CRF, CRF-PTX, and CTL-PTX rats. As shown in Fig. 3, brain microsomes purified from rats with CRF produced 50% less DFH ($P < 0.05$) compared with CTL, CTL-PTX, and CRF-PTX rats, suggesting that a decrease in CYP3A activity by CRF is likely due to impact of parathyroid-derived factor such as PTH.

CYP3A Expression in Astrocytes Cultured with Serum from CTL, CRF, and PTX Rats. To confirm the possible role of PTH present in uremic serum of CRF rats in the in vivo downregulation of brain CYP3A, we evaluated the effects of serum from control and CRF rats on cultured astrocytes isolated from normal rats. We incubated normal rat astrocytes for 48 hours with serum from control, CTL-PTX, CRF, or CRF-PTX animals. As shown in Fig. 4, serum from CRF rats reduced CYP3A expression by 30% ($P < 0.05$), whereas serum from CRF-PTX rats had no effects on CYP3A levels in rat astrocytes.

CYP3A Expression in Astrocytes Cultured with PTH. To provide direct evidence for role of PTH in modulating CYP3A levels in astrocytes, we cultured astrocytes isolated from normal rats in the presence of purified PTH. Results depicted in Fig. 5 show that incubation of astrocytes with 10 nM PTH decreased CYP3A by 30% ($P < 0.05$) compared with control astrocyte cultures lacking PTH, suggesting that CRF in rats may result in high serum PTH and that this could be an important factor in the downregulation of brain CYP3A in CRF rats.

Discussion

Brain cytochrome P450 plays an important role in the local metabolism of endogenous and exogenous compounds. Alteration in brain cytochrome P450 levels could significantly affect normal brain function or lead to local toxicity of drugs. It is a well recognized fact that CRF patients are subject to elevated drug toxicity and that P450 metabolism is impaired. The purpose of this study was to investigate the effect of CRF on brain P450 in whole-brain or specific regions of the brain of rats and the involvement of PTH. To address this, we measured P450 in cerebellum, cortex, hippocampus, and remaining brain tissue from CRF and CTL rats. To investigate the role of PTH, we measured CYP3A in whole-brain microsomes derived from CRF, CRF-PTX, CTL-PTX, and CTL rats. This study demonstrates that CRF affects the expression and activity of specific isoforms of brain P450s (CYP3A, 2C11, and 1A) and that PTH could be a mediator of this modulation.

Modulation of P450 described in this study is similar to that observed in the liver and intestine of CRF rats (Leblond et al., 2001, 2002). Indeed, in the different brain regions, we observed downregulation of CYP3A, CYP2C11, and CYP1A protein expression, but no modulation of CYP2D4 and CYP2E1 (Fig. 1). In rat liver and intestine, endogenous factors were shown to be responsible for the P450 impairment.

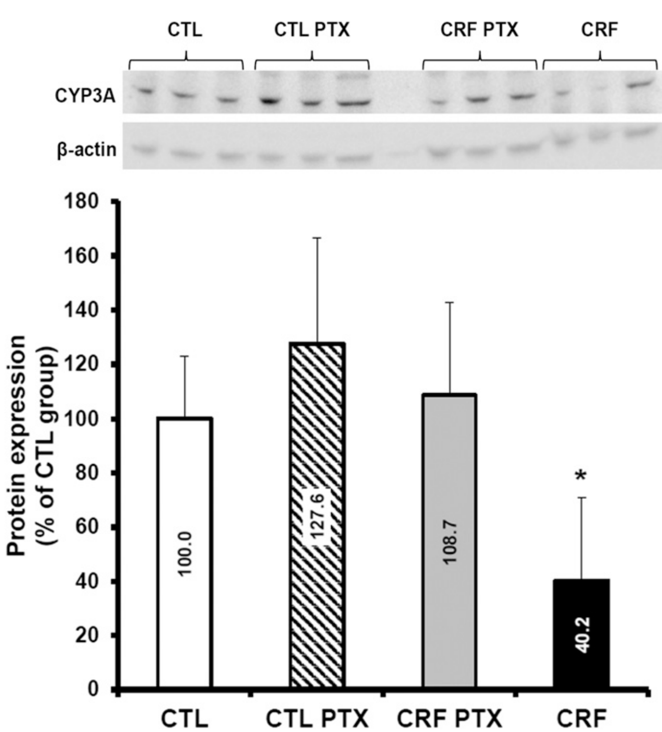

Fig. 2. CYP3A protein expression in whole-brain microsomes of CRF-PTX rats. Protein bands are expressed in densitometry units. The densitometry units measured for CYP3A in CTL (n = 13), CTL-PTX (n = 8), CRF-PTX (n = 11) and CRF (n = 13) rats were standardized by dividing them by the value obtained for β-actin. The mean relative quantity of control rats was arbitrarily defined as 100%. Graphic shows the mean expression in rats expressed as percent of controls ± S.D. *$P <$ 0.05 compared with control rats. Typical blot pictures are shown for CYP3A and the corresponding β-actin in each group.

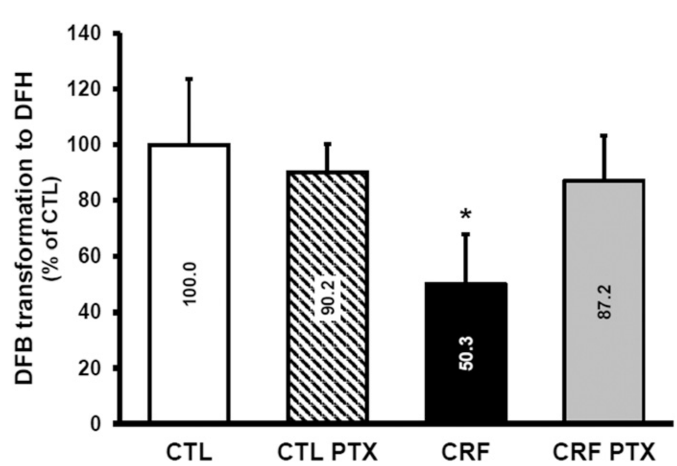

Fig. 3. CYP3A activity in whole-brain microsomes of CRF rats. CYP3A activity was measured by using DFB as a substrate and measuring its conversion to DFH in CTL (n = 13), CTL-PTX (n = 8), CRF (n = 13), and CRF-PTX (n = 11) rats. The mean relative DFH production in control rats was arbitrarily defined as 100%. Graphic shows the mean DFH production in rats expressed as percentage of controls ± S.D. *$P < 0.05$ compared with control rats.

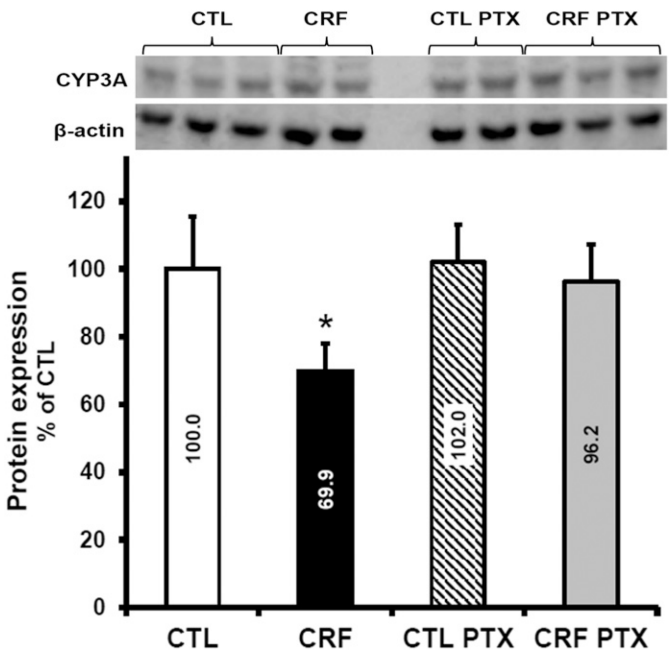

Fig. 4. CYP3A protein expression in astrocytes incubated with rat serum. Protein bands are expressed in densitometry units. The densitometry units measured for CYP3A in astrocytes incubated for 48 hours with serum from CTL (n = 5), CRF (n = 5), CTL-PTX (n = 4), and CRF-PTX (n = 4) rats were standardized by dividing them by the value obtained for β-actin. The standardized densitometry units of control condition were arbitrarily defined as 100%. Graphic shows the mean expression in rats expressed as percentage of controls ± S.D. *$P < 0.05$ compared with serum from control rats. Typical blot pictures are shown for CYP3A and the corresponding β-actin in each group.

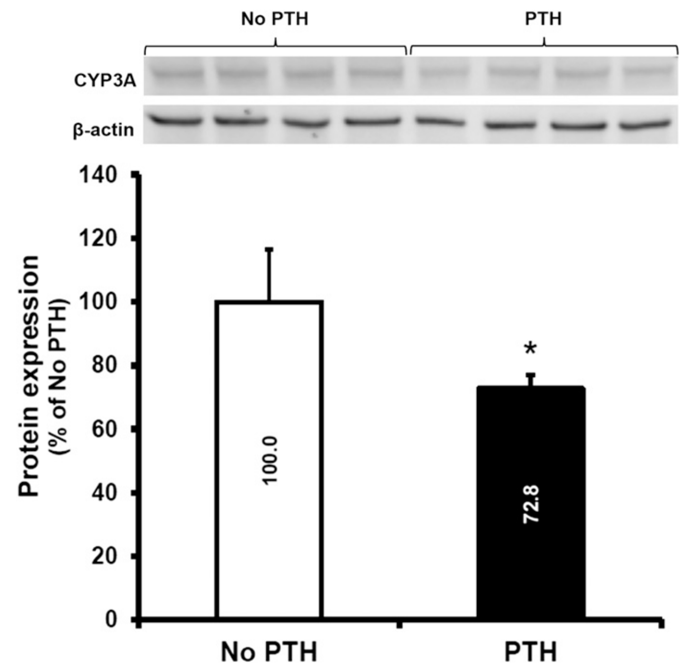

Fig. 5. CYP3A protein expression in astrocytes incubated with rat PTH. Protein bands are expressed in densitometry units. The densitometry units measured for CYP3A in astrocytes incubated for 48 hours without PTH (no PTH, n = 4) and with 10 nM PTH (PTH, n = 4) were standardized by dividing them by the value obtained for β-actin. The standardized densitometry units of no PTH were arbitrarily defined as 100%. Graphic shows the mean expression in rats expressed as percentage of no PTH ± S.D. *$P < 0.05$ compared with no PTH. Typical blot pictures are shown for CYP3A and the corresponding β-actin in each group.

Furthermore, in the liver, PTH was identified as the major mediator for the CYP3A modulation (Michaud et al., 2006, 2010).

To further develop our hypothesis that CRF may affect P450 levels in the brain via altered PTH levels, we first evaluated CYP3A expression in whole-brain microsomes from rats that underwent total parathyroidectomy. Whole-brain microsomes were used because P450 modulations had proven similar in all brain regions (Fig. 1) and because using whole-brain allowed for a higher sample yield. As shown in Table 3, PTX animals had undetectable PTH levels, whereas CRF animals had significantly increased levels compared with CTL animals. Also, CRF and CRF-PTX animals had similar renal impairment, as shown by their respective creatinine clearance rate. Figure 2 shows that, similar to that observed in rat liver (Michaud et al., 2006), parathyroidectomy abrogated the downregulation of CYP3A protein in whole-brain microsomes from CRF animals. PTX alone had no impact on CYP3A expression, as shown in the CTL-PTX group. In vitro, sera from CRF rats decreased CYP3A in normal rat astrocytes, whereas sera from CTL and PTX rats did not (Fig. 4). Also, incubation of astrocytes with 10 nM purified PTH for 48 hours led to a significant decrease in the protein expression of CYP3A in normal astrocytes (Fig. 5), supporting the hypothesis that PTH could be a factor in the downregulation of CYP3A protein expression in the rat brain in CRF, as previously shown in the liver (Leblond et al., 2001, 2002; Michaud et al., 2006).

Finally, we showed that not only CYP3A expression, but also its enzymatic activity, is reduced in whole-brain microsomes of CRF rats (Fig. 3). CYP3A enzymatic activity was assessed in whole-brain microsomes from CRF, CTL, CRF-PTX, and CTL-PTX rats by the in vitro conversion of DFB to DFH. This method was previously shown to be specific to rat CYP3A (Michaud et al., 2007). This mimics what was previously observed in the liver (Michaud et al., 2006).

Our results suggest that PTH may be a mediator in the downregulation of brain P450 by CRF in vitro and in vivo. Two PTH receptors are expressed in the brain: parathyroid hormone receptor 1 and 2, the latter having the highest expression (Goswami et al., 2014). Both receptors, in response to PTH binding, can increase cAMP production and $[Ca^{2+}]I$, and this can lead to activation of protein kinase A and phosphorylation of proteins, including P450s (Swarthout et al., 2002; Bisello et al., 2004). Phosphorylation of P450 by protein kinase A leads to a decrease in metabolic activity (Pyerin et al., 1987; Jansson et al., 1990). It can also lead to a downregulation of P450 gene expression (Sidhu and Omiecinski, 1995; Galisteo et al., 1999). Thus, there is a possibility that the PTH receptors in the brain are stimulated by the increased systemic PTH levels, which may in turn lead to P450 downregulation in CRF.

A major difference exists, however, between the previously cited reports in the liver and intestine (Leblond et al., 2001, 2002; Michaud et al., 2006) and our results: we found no effect of CRF on mRNA expression for the different P450 enzymes. This suggests a different pathway for the regulation of liver and brain P450 in CRF. Although significant progress has been made in our understanding of regulation of P450 in the brain and in different regions of the brain (Meyer et al., 2007; Miksys and Tyndale, 2013), our data indicate a possible different mode of P450 regulation in the brain compared with the liver. Our results point toward post-translational regulation, but the exact mechanisms involved remains to be identified.

This study clearly shows that CRF affects the expression of some P450 isoforms in the brain. These enzymes have endogenous and exogenous local functions. Whether altered P450 levels could lead to local drug toxicity or could affect normal brain metabolism remains to be studied.

Authorship Contributions

Participated in research design: Naud, Harding, Pichette.

Conducted experiments: Naud, Harding, Lamarche, Beauchemin, Leblond.

Performed data analysis: Naud, Lamarche, Leblond, Pichette.

Wrote or contributed to the writing of the manuscript: Naud, Leblond, Pichette.

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Address correspondence to: Dr. Vincent Pichette, Centre de recherche de l'Hôpital Maisonneuve-Rosemont, 5415 boul. de l'Assomption, Montréal, QC, Canada H1T 2M4. E-mail: vpichette.hmr@ssss.gouv.qc.ca