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Sex-dependent expression of CYP2C11 in spleen, thymus and bone marrow regulated by growth hormone

Chellappagounder Thangavel, Ravindra N. Dhir, Denys V. Volgin, Bernard H. Shapiro *

Laboratories of Biochemistry, Univeristy of Pennsylvania, School of Veterinary Medicine, 3800 Spruce Street, Philadelphia, PA 19104-6048, USA

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ABSTRACT

CYP2C11, the most commonly expressed isoform of cytochrome P450 in male rat liver, was measured in spleen, thymus and bone marrow by quantitative real-time PCR and enhanced Western blotting. CYP2C11 concentrations in the lymphoid tissues were a fraction of that observed in liver, but like the liver, were sexually dimorphic (M > F) with mRNA and protein levels in agreement. Although the response to hypophysectomy varied according to tissue and sex, expression levels of CYP2C11 in all measured tissues remained greater in males. Further differences in CYP2C11 expression between liver and lymphoid tissue were observed following restoration of the circulating masculine growth hormone profile in hypophysectomized rats. In contrast to the liver where the renaturalized growth hormone profile elevated CYP2C11 expression in both sexes, the response was opposite in spleen and thymus with isoform concentrations declining in both sexes. Lastly, the divergent response of CYP2C11 between the liver and immune system was examined in cultured splenocytes exposed to different mitogens. In contrast to the dramatic depletion of CYP2C11 reported in proliferating hepatocytes, mitogen-stimulation resulted in a significant elevation in splenocyte CYP2C11 expression. In summary, we report for the first time that thymus, spleen and bone marrow express, albeit nominal, sex-dependent levels of CYP2C11 (M > F) whose regulation appears to be under some hormonal control, but very different from that of the hepatic isoform.

1. Introduction

The cytochrome P450 monooxygenases are an ancient family of hemoenzymes that catalyze a large variety of essential metabolites, which include the synthesis and the deactivation of adrenal, gonadal and thyroid hormones, prostanoids, bile acids and fatty acids, as well as the detoxification of innumerable drugs and environmental chemicals. Hence their ubiquitous presence throughout all species where hundreds of isoforms have evolved to catalyze a countless number of substrates [1,2].

Whereas the liver clearly contains the highest content of total P450s, isoforms are expressed in kidney, gut, lung, skin, placenta, blood vessels and likely every cell in every organ system [1–3]. These extra hepatic tissues are often the target sites for therapeutic intervention against localized pathologies like cancer, infections and inflammations that coincidentally can result in toxicities arising from the biotransformation of the administered drug(s) [3,4]. Consequently, the content of tissue-specific P450 isoforms may represent an important parameter determining both the therapeutic efficacy and susceptibility to the toxic effects of administered xenobiotics.

* *Corresponding author*. Tel.: +1 215 898 1772; fax: +1 215 573 5187.
E-mail address: shapirob@vet.upenn.edu (B.H. Shapiro).
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In this regard, lymphatic tissue from spleen, thymus and bone marrow have been reported to express, albeit at considerably lower levels than observed in liver, isoforms from the CYP1A, CYP2A, CYP2B, CYP2E and/or CYP2J subfamilies [5–8].

CYP2C11 is the predominant isoform in male rat liver comprising >50% of the total P450 content of the organ [9]. Although female rats do not normally express CYP2C11, the isoform can be induced at substantial levels following hypophysectomy (HYPOX) and even more so when the HYPOX rats are infused with the masculine growth hormone (GH) profile [10]. Accordingly, in the present study we have examined lymphatic tissues for the presence of CYP2C11. Moreover, since hepatic expression of the isoform is sexually dimorphic due to sex differences in the circulating GH profiles, i.e., episodic for males, continuous-like for females [11,12], we compared splenic, thymic and bone marrow CYP2C11 levels in both male and female rats as well as determined the influence of GH on the sexually dimorphic response of the isoform.

2. Materials and methods

2.1. Animals

Animals were housed in the Univeristy of Pennsylvania Laboratory Animal Resources Facility under the supervision of certified laboratory animal medicine veterinarian. Animals were treated according to a protocol approved by the University's Institutional Animal Care and Use Committee. Intact and HYPOX male and female [Crl: CD (SD) BR] Sprague–Dawley rats were obtained from Charles River Laboratories (Wilmington, MA). Rats were HYPOX by the supplier at 8 weeks of age and maintained in our facility for 4–5 weeks on commercial rat pellets and 5% sucrose drinking water. Animals were housed under conditions of regulated temperature (20–23 °C) and photoperiod (12 h of light/12 h of darkness; lights on at 08:00 h). The effectiveness of the surgery was verified by the lack of body weight gain during the observation period and an absence of a pituitary or its fragments at necropsy.

2.2. Surgical implantation of catheters, GH treatment and assay

Indwelling right atrial catheters were implanted by methods described earlier [13,14]. After 3 days, the unrestrained and unstressed catheterized HYPOX rats were infused with 40 μg/kg b.w. of recombinant rat GH (National Hormone and Peptide Program and Dr. A.F. Parlow, Harbor-UCLA Medical Center, Torrance, CA) by an external syringe pump apparatus over a 3 min period with a frequency of six pulses per day (i.e., one pulse every 4 h) for a total of 14 pulses to replicate the physiologic masculine circulating GH profile [12,14]. Control rats were similarly infused with vehicle. After the sixth pulse, atrial blood samples (12 μl) were collected every 15 min for 6–7 continuous hours. Rat GH patterns were determined by radioimmunoassay [15]. Two to 3 h following the last GH pulse, rats were euthanized and livers, spleens and thymuses were quickly removed and minced into small pieces on ice-chilled Petri dishes. A fraction of minced liver was stored in RNA*later* (Ambion, Austin, TX) at −70 °C for RNA extraction.

2.3. Preparation of microsomes and whole cell lysate

Immediately after euthanizing, bone marrow was removed from the femur and tibia fibula. A single cell suspension, per rat, of bone marrow cells was prepared in RPMI-1640. Next, ACK buffer consisting of 150 mM NH_4Cl, 1 mM $KHCO_3$ and 1 μM EDTA, was used to lyse the red blood cells present in the bone marrow suspension. The bone marrow cells were pelleted and stored at −70 °C for future use. Whole cell lysate from the bone marrow pellets was prepared by resuspending in lysis buffer containing 50 mM Tris–HCL (pH 7.5), 0.3 M NaCl, 1% Triton X-100, 5 mM EDTA and 0.5% Nonidet P-40. The crude extract was passed through a 22-gauge needle 10 times at 4 °C before centrifugation at 12,000 × *g* for 20 min. The supernatant (whole cell lysate) was then removed and stored at −70 °C for further analysis. Microsomes from liver, spleen and thymus were prepared as described earlier [15].

2.4. In vitro exposure to mitogens

Spleens were removed aseptically from intact male rats. Splenocytes were prepared by mincing the spleen and passing through a 70G stainless steel mesh to make a single cell suspension. Collected cells were washed twice with complete medium and plated into six well culture plates; ~10^6 cells/well. Cells were incubated in a humidified incubator at 37 °C under an atmosphere of 5% CO_2/95% air in RPMI-1640 medium containing either phytohaemagglutinin (PHA), 5 μg/ml; concanavalin A (CONA), 5 μg/ml; bacterial lipopolysaccharide (LPS), 25 μg/ml; or diluent. After 4 days, cell proliferation was determined by modification of the CellTiter assay according to the manufacturer (Promega, Madison, WI), after which cells were harvested for RNA extraction.

2.5. Western blotting

Microsomal fractions of liver, spleen and thymus as well as whole cell lysates of bone marrow were electrophoresed under denaturating conditions on 1.5 mm thick, 10% SDS–PAGE gels as we described previously [16]. The blots, incubated in Super-Signal West Femto (Pierce, Rockford, IL), were analyzed with an Alpha Innotech FluorChem 8800 gel documentation system using a visible light source (San Leandro, CA). Densitometric units were obtained as integrated density values as calculated by the software supplied with the gel documentation system. Equal loading of protein was confirmed by using Ponceau S staining and Western blot analysis for the expression of β-actin. Furthermore, protein values were normalized to two control samples repeatedly run on all blots.

2.6. RNA extraction

Whereas liver, spleen and thymus were homogenized with Trizol reagent for RNA extraction according to the manufacturer's recommended protocol (Life Technologies, Carlsbad, CA), the bone marrow cell pellet was first passed through a 21G needle at least 10 times in the presence of Trizol, thereafter

extracted according to the same procedure. The samples were treated with DNAse. The RNA was quantified spectrophotometrically at 260 nm and purity was confirmed by the 260/280 nm ratio. Total RNA (20 μg) was separated on agarose gel containing 2.2 M formaldehyde to confirm its integrity as assessed by the intensities of the 28S and 18S rRNA bands visualized after ethidium bromide staining.

2.7. Reverse transcription (cDNA synthesis)

cDNA synthesis was conducted with 1 μg of DNA free total RNA in a 20 μl reaction mixture containing 1× reverse transcriptase (RT) buffer, 5 mM $MgCl_2$, 1 mM of each dNTPs, 1 unit of RNase inhibitor, 2.5 units of MuLV reverse transcriptase (Promega, Madison, WI), 2.5 μM oligo $d(T)_{16}$ (Applied Biosystem, Foster City, CA). The mixture was incubated at 42 °C for 1 h and stored at −20 °C. Appropriate RT (−) controls were included in this study to confirm the absence of genomic DNA.

2.8. Semi-quantitative PCR

The PCR reaction was carried out using a Perkin-Elmer Gene Amp PCR system 2400 in a 100 μl reaction volume containing 1× PCR buffer, 2 mM $MgCl_2$, 2.5 units of Taq DNA polymerase, 1 μl of cDNA and 150 nM of CYP2C11 forward and reverse primers. The primer sequences and cycling conditions for CYP2C11 [17] and tubulin [18] have been reported earlier and are presented as Supplementary data. The PCR amplified products were separated on 1.5% agarose gels run in 0.5× TBE buffer for 90 min at 80 V. The DNA gel was stained with ethidium bromide for 20 min (0.1 μg/ml) and the DNA band intensities were quantified with a Alpha Innotech FluorChem 8800 gel documentation system using a UV lamp. Densitometric units were obtained as integrated density values as calculated by the software supplied by the manufacturer. CYP mRNAs were normalized to total RNA levels for individual livers. The PCR products were purified and sequenced with the DNA sequencer model 377 (Applied Biosystems, Foster City, CA) using the specific primers for CYP2C11. According to a Blast search (www.ncbi.nlm.nig/gov), the purified PCR products exhibited 100% sequence homology with their respective CYP genes (*Rattus norvegicus*) (sequences not presented).

2.9. Quantitative PCR (real-time PCR)

The quantitative real-time PCR reactions were conducted, as we reported earlier [19], in a total 20 μl reaction mixture containing 2.5 μl of SYBR Green I (Molecular Probes, Eugene, OR), 250 μM of dNTPs, 250 nM of each primers, 0.4 μl of DMSO, 0.6 μl of titanium Taq DNA polymerase (BD Biosciences, Palo Alto, CA), 1 μl of cDNA (reverse transcriptase product) and CYP2C11 [17] and tubulin [18] primer sets (reverse and forward). PCR cycling was performed in glass capillaries using the Light Cycler rapid thermal cycler system (Indianapolis, Roche, IN) as per manufacturer's instructions. The final PCR products were subsequently melted by linear heating (0.2 °C/s) to 95 °C. The position and size of the melting peak provided an assessment of the generation of expected cDNAs, the presence of which was confirmed by agarose gel electrophoresis. For the quantification of cDNA copy numbers in the samples, a calibration curve was generated from serially diluted cDNA samples containing known copy numbers of the target gene. These samples were generated from the gel purified PCR product and quantified by densitometry as described previously [18].

The PCR product for CYP2C11 was purified and sequenced with the DNA sequencer model 377 (Applied Biosystem, Foster City, CA) using the specific primer for CYP2C11. According to a Blast search (www.ncbi.nlm.nig.gov) the purified PCR product exhibited 100% sequence homology with the CYP2C11 gene (*R. norvegicus*) (sequence not presented).

2.10. Statistics

All the data were subjected to analysis of variance. Significant differences were determined with *t* statistics and the Bonferroni correction for multiple comparisons.

3. Results

3.1. Sexually dimorphic expression of CYP2C11 in hematopoietic tissue

As expected [11,12], we observed a profound sexual dimorphism in hepatic CYP2C11 mRNA in which concentrations in intact female liver were <0.2% of that in intact male liver (Table 1);

Table 1 – CYP2C11 and tubulin mRNAs in hematopoietic tissues of intact and hypophysectomized (HYPOX) male and female rats

	Liver		Thymus		Spleen		Bone marrow	
	Male	Female	Male	Female	Male	Female	Male	Female
CYP2C11								
Intact	640473 ± 92232	1106 ± 175*	2380 ± 462	2703 ± 490	987 ± 254	493 ± 184*	1040 ± 449	124 ± 21*
HYPOX	83823 ± 14429†	36472 ± 8189*,†	1850 ± 398	1048 ± 347*,†	893 ± 63	313 ± 27*	21006 ± 5203†	655 ± 163*,†
Tubulin								
Intact	24.6 ± 3.5	16.1 ± 3.1*	2321 ± 281	4538 ± 643*	370 ± 108	308 ± 88	3.0 ± 1.8	1.6 ± 0.5
HYPOX	49.5 ± 14.4†	45.0 ± 2.7†	2322 ± 32	2058 ± 160*,†	556 ± 116	601 ± 136†	72.1 ± 27.3†	85.0 ± 40.0†

Total copies of CYP2C11 and tubulin (the latter × 10^3) mRNA/μg total RNA determined by quantitative real-time PCR.
Mean ± S.D.
* $P < 0.01$ when females are compared to males with the same treatment.
† $P < 0.01$ when HYPOX rats are compared to intact rats of the same sex.

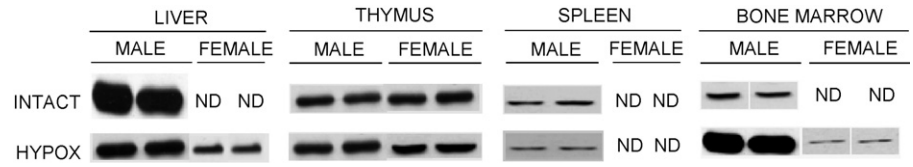

Fig. 1 – CYP2C11 protein levels in hematopoietic tissues. Western blot analysis was performed using microsomes from liver (2 μg protein), thymus, spleen and whole cell lysate of bone marrow (40 μg protein) obtained from intact and hypophysectomized (HYPOX) male and female rats. Bands are representative of an $n \geq 5$. ND, not detected.

explaining why studies using less sensitive assays than quantitative real-time PCR report undetectable levels of the isoform in female rat liver [10,11]. Although not as dramatic as what could be characterized as the ''male-specificity'' of CYP2C11 mRNA in liver; spleen and bone marrow exhibited a ''male-predominance'' in transcript levels in intact rats. Concentrations of the isoform were two- and eight-fold greater in male spleen and bone marrow, respectively. There was no statistical sex difference in CYP2C11 mRNA in the thymus of intact rats (Table 1). Regardless of the sex differences in hematopoietic tissue CYP2C11 mRNA, the magnitude of the concentration differences between lymphoid tissues and liver in intact rats was enormous. Concentrations of the transcript in thymus, spleen and bone marrow were in the range of that observed in intact female liver, but generally hundreds of times lower than found in male liver.

The sexual dimorphisms and comparative concentrations of CYP2C11 mRNA in the hematopoietic tissues were similarly reflected at the protein level of intact rats (Fig. 1). Not surprisingly, Western blotting, even when using an extremely sensitive enhanced chemiluminescent substrate, was considerably less sensitive than real-time PCR. Nevertheless, we observed an extreme male specificity of CYP2C11 protein in liver and a still considerable male predominance of the protein in spleen and bone marrow. In agreement with the mRNA findings, concentrations of the cytochrome P450 protein were hundreds of times greater in male liver as compared to the other hematopoietic tissue. (Note that the concentration of hepatic microsomes used in the Western blots was only 5% of that probed from the other tissues; see Fig. 1 caption.)

3.2. *Persistent sexually dimorphic expression of CYP2C11 in hematopoietic tissue after hypophysectomy (HYPOX)*

There are two notable observations regarding the effects of HYPOX. First, the sexually dimorphic expression of CYP2C11 in which M > F persisted in the HYPOX state (Table 1, Fig. 1). In fact, HYPOX induced sex differences in thymic levels of the isoform that were not apparent in the intact animals. Second, the direction of change in CYP2C11 mRNA and protein expression (i.e., increase/decrease) resulting from HYPOX varied with both sex and tissue. Whereas hepatic concentrations of the transcript declined by >7-fold in males following HYPOX, bone marrow levels responded with a 20-fold mRNA increase in males. CYP2C11 mRNA in the thymus and spleen of males was unaffected by pituitary ablation. In the case of females, CYP2C11 mRNA was increased in liver and bone marrow ~30- and ~5-fold, respectively, and decreased ~60% in thymus following HYPOX.

Thus, HYPOX had no effect, regardless of sex, on splenic CYP2C11; reduced isoform concentrations only in female thymus and male liver and dramatically increased levels in the bone marrow of both sexes as well as female liver.

3.3. *Sexually dimorphic expression of tubulin*

Because it is generally assumed that expression levels of so-called ''housekeeping'' genes are stable, they are used as internal controls to normalize expression levels of treatment-responsive genes. However, as apparent from Table 1, concentrations of tubulin mRNA measured by quantitative PCR can vary rather widely according to tissue, sex and/or hormonal state (e.g., HYPOX). These findings are in agreement with our [19] as well as other [20,21] earlier reports of significant sex, hormonal and non-hormonal effects on the expression levels of such housekeeping genes as cyclophilin, tyrosine aminotransferase, β-actin, tubulin, glyceraldehyde-3-phosphate dehydrogenase and 18S in rat liver [19]. Accordingly, we normalized real-time PCR determined CYP2C11 mRNA concentrations to total RNA (Table 1) as we [22] and others [23] have reported.

3.4. *Renaturalization of the circulating episodic rat GH profile*

The pattern of circulating GH observed from serial blood samples collected from control (intact) rats infused with rat GH-vehicle (Fig. 2) exhibited typical sexually dimorphic GH profiles [10,12,15]. Intact males secreted GH in episodic bursts (~250 ng/ml of plasma) about every 3.5–4 h. Between the peaks, GH levels were undetectable. In intact females the hormone pulses occurred more frequently and irregularly and were of lower magnitude than those in males, whereas the interpulse concentrations of GH were always measurable. HYPOX completely eliminated any detectable levels of circulating GH (not shown). Intra-atrial infusion of male and female HYPOX rats with 40 μg rat GH/kg b.w. every 4 h produced an episodic circulating GH profile that was near identical to the hormone profile observed in intact male rats (Fig. 2).

3.5. *CYP2C11 expression in hematopoietic organs of HYPOX male and female rats infused with the masculine GH profile*

Because the masculine episodic GH profile is the sole endogenous regulator of hepatic CYP2C11 [10,11,24], it was not surprising that the renaturalization of the profile in HYPOX

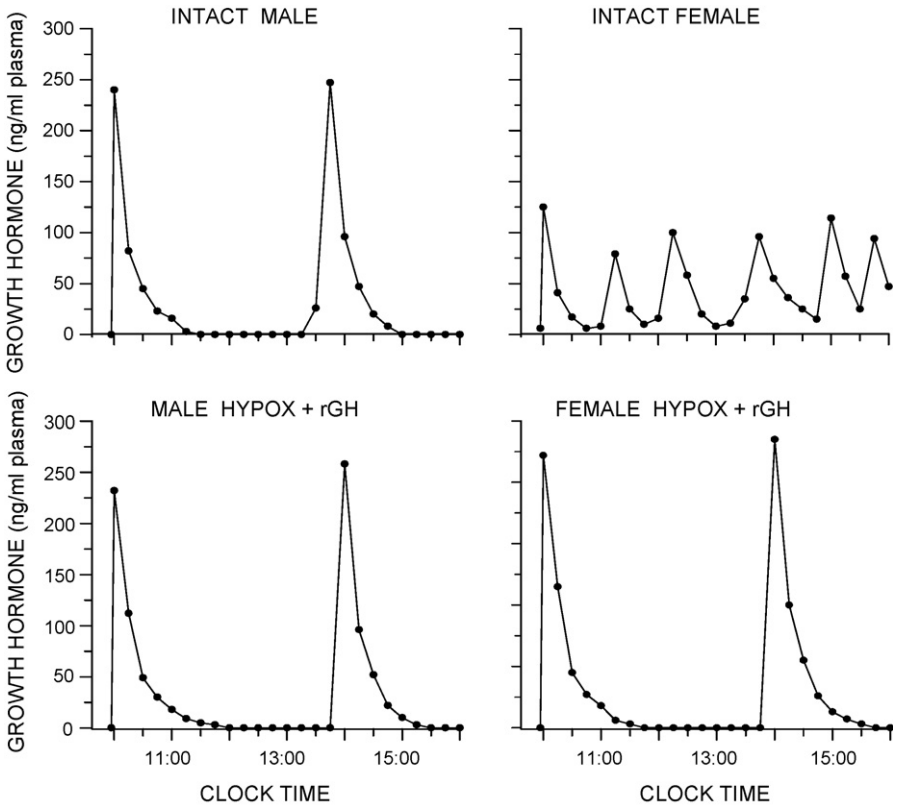

Fig. 2 – Plasma levels of circulating growth hormone obtained from individual undisturbed catheterized intact and hypophysectomized (HYPOX) episodic rat growth hormone (rGH)-treated male and female rats. Serial blood samples were obtained from vehicle-infused intact and rGH-infused HYPOX rats after the sixth intra-atrial injection of 40 μg of rGH/kg b.w. Plasma was collected from the intact rats for 7 consecutive hours to allow the rGH peaks to be aligned with the peaks of the infused HYPOX rats. Similar findings were obtained from at least five additional rats in each treatment group.

male and female rats induced a significant elevation of the hepatic isoform in both sexes (Fig. 3).[1] The sex differences (M > F) in thymic and splenic CYP2C11 mRNA in HYPOX rats remained statistically significant even when measured by the less sensitive semi-quantitative PCR (Fig. 3). However, in contrast to the hepatic isoform, renaturalization of the episodic GH profile reduced CYP2C11 expression in the thymus and spleen of both sexes of HYPOX rats; albeit considerably more so in the spleen than the thymus.

3.6. *In vitro response of splenocyte CYP2C11 to mitogens*

After 4 days in culture without any proliferative agents, splenocyte CYP2C11 mRNA levels declined ~65% (Fig. 4). In contrast, the addition of mitogens to the medium resulted in the expected proliferation (data not presented) ameliorating the culture-induced decline in expression levels of the isoform. In fact, LPS, the well-known B-cell mitogen [25], completely prevented the loss of splenocyte CYP2C11 mRNA while PHA and CONA, specific T-cell mitogens [26], reduced the in vitro loss of the isoform by ~50% (Fig. 4).

[1] The renaturalized episodic GH profile did not completely restore hepatic CYP2C11 to pre-HYPOX levels because the liver likely needs to be exposed to the profile for a longer time period in order to fully induce male-like levels of the isoform [24,39].

4. Discussion

Although CYP2C isoforms are the predominant P450s in rat liver [9–11], their presence in lymphatic tissue has not been established. Our application of highly sensitive real-time PCR and enhanced Western blotting has identified CYP2C11 in thymus, spleen and bone marrow at transcript and protein levels that were in agreement. Thymic testosterone 2α-hydroxylase, a specific measure of CYP2C11 activity [27], was reported in male rats to be ~0.2% of hepatic activity [7], which is very similar to what be observed measuring CYP2C11 mRNA. Otherwise there appears to be no other reports identifying CYP2C11 in lymphoid tissue.

Considering the omnipresence of sexual dimorphisms at every level [28], it is not surprising that CYP2C11 concentrations in hematopoietic tissues are also sex-dependent. The male-specificity of the hepatic isoform has been widely reported [11,12,24,27]. Although we found no sex differences in thymic CYP2C11 in intact rats, there was a substantial sexual dimorphism in the spleen (M:F, 2:1) and bone marrow (M:F, 8:1). As observed in all the tissues, and now including the thymus, the sexual dimorphism in CYP2C11 expression in hematopoietic tissues (M > F) was retained following HYPOX. However, the response was tissue variable. In males, hepatic isoform concentrations declined (~85%), thymic and splenic levels were unchanged and bone marrow levels increased

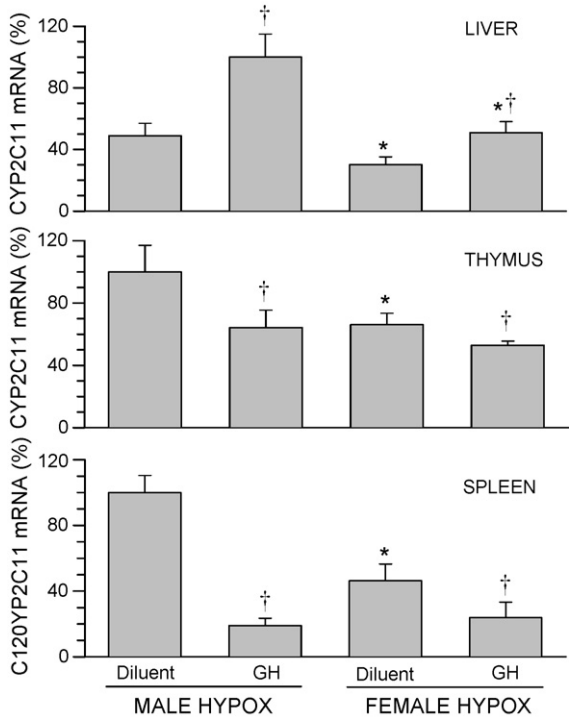

Fig. 3 – Hepatic, thymic and splenic CYP2C11 mRNA from hypophysectomized (HYPOX) and growth hormone (GH)-restored male and female rats. The physiologic masculine episodic GH profile was restored to HYPOX male and female rats by infusing rat GH (40 μg/kg b.w.) via an indwelling atrial catheter attached to an external pumping apparatus, as a 3 min pulse every 4 h for 14 pulses. Control rats received GH-diluent. CYP2C11 mRNA was determined by semi-quantitative PCR. Values are presented as a percentage of CYP2C11 mRNA with the highest value in each panel arbitrarily designated 100%. Each data point is a mean ± S.D. of at least five animals for each treatment. *$p < 0.01$, when comparing males and females with the same treatment. †$p < 0.01$, when comparing GH effect in the same sex.

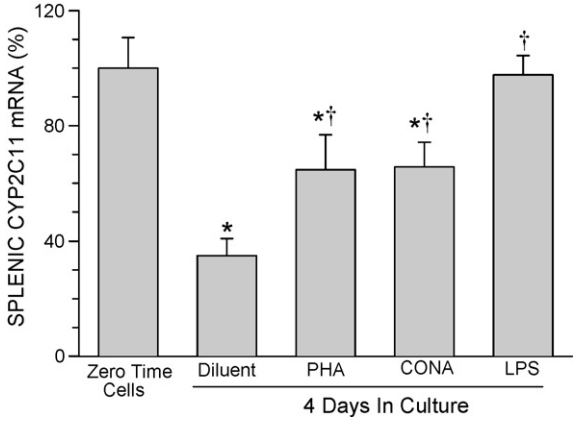

Fig. 4 – Response of splenic CYP2C11 mRNA exposed to various mitogens. Hypophysectomized male splenocytes were harvested and either analyzed immediately before plating (zero time cells) or cultured for 4 days in complete media containing either phytohemagglutinin (PHA), concanavalen A (CONA), bacterial lipopolysaccharide (LPS) or diluent. CYP2C11 mRNA was determined by semi-quantitative PCR. Sufficient viable cells were isolated from a single spleen for all treatments presented in the figure. Values are presented as a percentage of CYP2C11 mRNA in splenocytes from zero time cells arbitrarily designated 100%. Each data point is the mean ± S.D. of at least five animals for each treatment. *$p < 0.01$, when compared to zero time cells. †$p < 0.01$, when compared to cultures exposed to diluent.

(~20-fold) following HYPOX. In contrast, CYP2C11 expression in HYPOX females increased in liver (~19-fold) and bone marrow (~5-fold), was unchanged in spleen and declined in thymus (~60%). Whereas these findings indicate hormonal regulation of CYP2C11 expression in the hematopoietic tissues, the response was highly variable between tissues, suggesting no common mechanism of hormone regulation.

GH, and in particular, the masculine episodic circulating profile, is the sole endogenous regulator of hepatic CYP2C11 expression. The feminine continuous GH profile suppresses CYP2C11; hence its near absence in female liver. GH ablation (e.g., HYPOX) allows for minimal levels of expression [11,12,24,27]. Circulating lymphocytes as well as those in the thymus, spleen and bone marrow secrete GH where it appears to act as an autocrine/paracrine regulator of the immune system [29–31]. GH stimulates thymocyte proliferation and differentiation, as well as the activation and proliferation of peripheral T- and B-cells [32]. Natural killer cell activity is reduced in patients with GH deficiency [33] and restored by administered GH [34]. GH potentiates erythropoiesis [35], lymphopoiesis [36] and granulopoiesis [37]. The hormone can augment the production of superoxides by macrophages [38]. Exposure to viruses, as well as environmental and tumor-derived antigens can stimulate the local secretion of GH by lymphocytes [30]. In this regard, the regulation of GH secretion is very different between the endocrine and immune systems [30]. Nevertheless, since (1) GH is the sole endogenous regulator of hepatic CYP2C11, (2) thymus, spleen and bone marrow express CYP2C11, (3) these lymphoid tissues contain dramatic concentrations of lymphocytes and (4) lymphocytes secrete and are themselves regulated by local GH, it seemed reasonable that GH might normally regulate expression of the isoform in the immune system.

Although we were unable to block lymphocyte-derived GH, HYPOX completely eliminated any detectable concentrations of the hormone from the circulation. Because CYP2C11 expression was greater in males, and the masculine GH profile regulates hepatic CYP2C11, we chose to investigate the effects of the restored episodic hormone profile on the isoform in hematopoietic tissues. Infusion of the physiological masculine episodic GH profile (which is much more effective than the commonly used intermittent injections [39]) of rat GH to HYPOX rats significantly induced hepatic expression of CYP2C11 in both sexes (albeit less so in females who are inherently less responsive to the masculine GH profile [10]). In contrast, restoration of the episodic GH profile had a

suppressive effect on thymic and splenic CY2C11 expression in both sexes suggesting a substantial difference in the regulation of the isoform by GH between the liver and that of the thymus and spleen. In this regard, CYP2E1 activity in liver is increased by GH [12,15], but in lymphoma cells was found to be suppressed by GH [40]. Incidentally, the fact that circulating GH (i.e., pituitary) can regulate CYP2C11 expression in thymus and spleen does not preclude, but rather supports the possibility that lymphocyte-derived GH may also regulate expression of the isoform in the immune system.

The opposite response of CYP2C11 in the liver to that of the lymphoid tissues to episodic GH was further investigated by examining the effects of mitogens on splenic CYP2C11 expression. Similar to hepatocytes in culture [41], CYP2C11 levels were rapidly depleted in splenocytes cultured in basic media without inducers. Hepatocyte proliferation resulting from regeneration [42] or exposure to mitogens [43] suppresses transcription of adult isoforms of P450, including CYP2C11. In contrast, CYP2C11 expression in cultured splenocytes could be restored to pre-isolation (whole organ) levels by the addition of mitogens to the media. Thus, whereas hepatic P450s are lost during proliferation, requiring mature cells for expression, expression of the isoforms (or at least CYP2C11) are actually increased in the proliferating splenocyte.

Our results indicate that regulation, particularly hormonal, of CYP2C11 in hematopoietic tissues is rather complex. There is, however, precedence from the complicated regulation of a near dozen sex-dependent hepatic P450s where GH can either induce, suppress or may have no effect on each isoform depending upon the hormone's secretory profile and the animal's sex [10,39,44]. Thus, whereas restoration of the circulating masculine episodic GH profile to HYPOX rats suppresses thymic and splenic CYP2C11 expression, it induces hepatic CYP2C11 but suppresses hepatic CYP2A2 and CYP3A2 [24,39].[2] The effects of HYPOX are also complex. It appears that CYP2C11 expression in liver, thymus, spleen and bone marrow, also influenced by sex, responds very differently to HYPOX. Since HYPOX depletes the body of at least a dozen hormones, including, not just GH, but those of the thyroid, adrenal and gonads, it is not possible without far more experiments, to determine which hormones may induce or suppress CYP2C11 in the immune system of both male and female rats. In this regard, thyroid hormone is required for full expression of several hepatic P450s [45], androgens are the primary regulators of kidney CYP2C11 in rats [46] and hepatic P450-dependent enzymes in chickens [47] and glucocorticoids are essential for expression of human CYP3A4 [48].

Perhaps these seemingly innumerable pathways regulating P450 expression in different species and tissues can be understood by considering that the cytochrome P450s are an ancient enzyme family whose origins go back more than 2.5 billion years [1], long before the endocrine system evolved [49]. It is not surprising then, that various isoforms evolved taking on different functions in different organisms and in different tissues under a multitude of regulatory controls. In fact, regulation of the immune system by GH locally secreted by lymphocytes represents the most primitive and ancient method of hormone delivery, i.e., autocrine/paracrine, which very much predates the origins of the endocrine system [50]. In agreement, regulation of lymphocyte GH secretion, unlike that of the more recently evolved vertebrate pituitary, is independent of neuroendocrine feedback [30].

Lastly, the function(s) of CYP2C11, or for that matter, any of the previously identified P450 isoforms in the immune system, is unknown. Any conclusions would be speculative and would have to consider the abilities of the enzymes to both activate and deactivate a broad range of metabolites. However, even in the case of hepatic CYP2C11, clearly the most highly expressed isoform in male rat liver [9], we only know what the enzyme is capable of metabolizing, but not necessarily what is its physiologic function.

In summary, thymus, spleen and bone marrow express nominal, sex-dependent levels of CYP2C11 (M > F) whose regulation appears to be under some hormonal (including GH) control, but very different from that of the hepatic isoform.

[2] The fact that CYP2C11 expression in liver and immune organs are regulated differently by GH is understandable considering that the isoform likely catalyzes different substrates, performing different functions in various tissues, and thus requiring dissimilar modes of regulation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bcp.2007.07.035.

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