Hormonal Regulation of Microsomal Cytochrome P450 2C11 in Rat Liver and Kidney

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ABSTRACT

The current study was conducted to investigate hormonal regulation of cytochrome P450 2C11 (CYP2C11) in rat liver and kidney of adult male rats. In two experiments, hypophysectomy (Hx) resulted in decreased (P < .05) hepatic CYP2C11 apoprotein and mRNA levels. Growth hormone (GH) replacement of Hx rats prevented the decline in hepatic CYP2C11 apoprotein and mRNA levels, whereas, subcutaneous injection of testosterone had no effect. Rat pituitary extract is equally effective in intact or castrated Hx rats in preventing the decline in hepatic CYP2C11 apoprotein and mRNA levels. Specific neutralization of rat GH by sheep anti-rat GH serum reduced (P < .05) serum IGF-I concentrations, hepatic CYP2C11 apoprotein and mRNA levels. Hx of male rat resulted in decreased (P < .05) renal CYP2C11 apoprotein and mRNA levels, and treatment with GH failed to prevent these effects; however, supplementation of Hx rats with testosterone or rat pituitary extract prevented the Hx-induced decrease of renal CYP2C11 apoprotein and mRNA levels, and the effects of rat pituitary extract occurred only in intact rats. Neutralization of rat GH by anti-rGH significantly reduced (P < .05) CYP2C11 mRNA levels and serum T concentrations but not serum LH concentrations. These results indicate that although hepatic CYP2C11 is regulated by GH, rat renal CYP2C11 is regulated primarily by gonadal steroids.

The cytochromes P450 constitute a large family of heme-containing proteins that play an important role in the biotransformation of various xenobiotics and endogenous compounds (Nelson et al., 1993). CYP2C11 accounts for approximately one third of the total cytochrome P450 in male rat liver and is essentially undetected in female rat liver (Kato and Yamazoe, 1990). CYP2C11 metabolizes a host of xenobiotics such as benzphetamine, aminopyrine, ethylmorphine, benzo[a]pyrene and warfarin (Guengerich et al., 1982), and it is responsible for stereospecific metabolism of endogenous steroids. For example, 2α- and 16α-hydroxylation of testosterone (T) and 16α-hydroxylation of estradiol are catalyzed by CYP2C11 (Ronis et al., 1996; Waxman, 1988). The constitutive male-specific CYP2C11 expression in rat liver is developmentally up-regulated at the transcriptional level by the male pattern of growth hormone (GH) secretion (Legraverend et al., 1992).

It is well known that GH secretion in rats is pulsatile (Tannenbaum and Martin, 1976) and the plasma GH pulse profile is sexually dimorphic and modulated by gonadal steroids (Millard et al., 1987). The male pattern of GH secretion is reported to be responsible for male specific expression of rat hepatic CYP2C11, whereas the female GH secretory pattern suppresses hepatic CYP2C11 expression and positively regulates hepatic CYP2C12 expression (Janeczko et al., 1990; Kato et al., 1986; MacGeoch et al., 1985; Morgan et al., 1985; Waxman et al., 1991). GH pulse profile consists of at least three important components, namely, pulse amplitude, pulse frequency, and interpulse interval, that are thought to be important signals for gene expression. Thus, treatments that significantly alter these signals of male rat GH may be expected to affect CYP2C11 expression. We have demonstrated that chronic ethanol treatment resulted in a disruption of GH secretion and reduced serum T concentrations as well as hepatic CYP2C11 apoprotein and mRNA levels (Badger et al., 1993), so the inhibition of hepatic CYP2C11 after chronic ethanol treatment in rats is thought to be at least in part due to the change of GH secretory pattern of male rats to a pattern that resembles that of female rats.

Most of what has been reported in the literature on GH-regulated CYP2C11 expression is based on the Hx rat model, in which multiple hormone deficiencies make data interpretation difficult (Janeczko et al., 1990; Kato et al., 1986; Mor-

ABBREVIATIONS: GH, growth hormone; rGH, rat growth hormone; HGH, human recombinant growth hormone; LH, luteinizing hormone; Pit, rat pituitary extract; T, testosterone; Hx, hypophysectomy, hypophysectomized; IGF-I, insulin-like growth factor-I; sham, sham-hypophysectomized animals; C, hypophysectomized plus castrated animals; intact, unoperated animals; s.c., subcutaneous; ADU, arbitrary densitometric units.

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studied CYP2C11 regulation in rat kidney and compared it with the liver counterpart in Hx male rats treated with hGH, rGH, Pit and T. We also report for the first time the effects of GH on the regulation of CYP2C11 in rat liver as well as in kidney by treating rats with sheep anti-rGH serum (Madon et al., 1986), which specifically neutralizes rat GH.

Methods

Animals. Adult male Sprague-Dawley rats (8 weeks of age; Hx, sham, C, or intact groups) were purchased from Harlan Industries (Indianapolis, IN). Operated animals were allowed to recover for 4 days after surgery, weighed and randomly assigned to experimental groups. They were housed individually and maintained under constant temperature (24°C) and a 12-hr light/dark cycle in an AAA-LAC-approved animal facility. All rats had ad libitum access to standard rat food and either water or an solution containing 35 mM NaCl, 1.1 mM KCl, 0.32 mM CaCl₂ and 0.09 mM MgCl₂ throughout the experiment (Terry and Crowley, 1980).

Hypophysectomy, castration and hormone replacement experiment. Replacement of operated rats with hormone or vehicle began 4 days after surgery. rGH (1.8 IU/mg; National Hormone and Pituitary Program) and Pit were dissolved in or diluted with 0.9% NaCl, 1.1 mM KCl, 0.32 mM CaCl₂ and 0.09 mM MgCl₂ and administered to the Hx rats by s.c. injection twice daily at the doses of 133 and 50 μg rGH (expressed as RP-2) per injection, respectively. hGH (2.6 IU/mg; Genentech, South San Francisco, CA) was dissolved in 0.9% saline solution and administered to the Hx rats by either s.c. injection (92 μg/injection) twice daily or continuous infusion using a subcutaneously implanted osmotic minipump (Alza, Palo Alto, CA) at the rate of 7.7 μg/hr. T in corn oil was given to rats by daily s.c. injection at the dose of 3 mg/kg body weight. In experiment 1, seven groups of animals were studied: (1) sham-Hx rats receiving twice-daily s.c. injections of

<table>
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<tr>
<th>Treatment</th>
<th>Body weight gain</th>
<th>Serum IGF-I</th>
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<tr>
<td>g</td>
<td>ng/ml</td>
<td></td>
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<tr>
<td>Sham + NS</td>
<td>49.9 ± 2.2a</td>
<td>1440 ± 35a</td>
</tr>
<tr>
<td>Hx + NS</td>
<td>0.2 ± 2.7b</td>
<td>41 ± 2b</td>
</tr>
<tr>
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<td>38.7 ± 2.2a</td>
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<tr>
<td>Hx + rGH(p)</td>
<td>48.2 ± 2.1a</td>
<td>N.D.</td>
</tr>
<tr>
<td>Hx + Pit(p)</td>
<td>49.7 ± 6.6b</td>
<td>N.D.</td>
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<tr>
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<td>41.2 ± 2.4b</td>
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<tr>
<td>Hx + T</td>
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<tr>
<td>Hx + C + T</td>
<td>14.0 ± 2.7b</td>
<td>N.D.</td>
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Adult male rats were sham-hypophysectomized (Sham), hypophysectomized (Hx) or Hx plus castrated (C) at 8 weeks of age. Hormone replacement and vehicle treatment were started 4 days after operation. NS, 0.9% saline solution; hGH(p), 92 μg hGH/injection; rGH(p), 133 μg rGH/injection; Pit(p), 50 μg rGH in rat pituitary extract/injection; T, 3 mg/kg/day testosterone. All hormones except T, which was delivered by once-daily s.c. injection, were delivered by s.c. injection twice daily for 7 days. Controls received vehicle (NS) injection. Values are the mean ± S.E.M. of 5 to 10 rats. N.D., not determined. Values bearing different superscripts differ by P < .05.

Fig. 1. Effects of Hx and hormone replacement on hepatic CYP2C11 apoprotein and mRNA levels. Adult male rats were sham-Hx (sham) or Hx (Hx) at 8 weeks of age. Hormone replacement and vehicle treatment were started 4 days after Hx as follows: (1) twice-daily s.c. injection of 92 μg of hGH [hGH(p)] or Pit [Pit(p)] containing 50 μg of rGH per injection for 7 days or (2) continuous infusion of hGH via an s.c. implanted osmotic minipump for 7 days [hGH(i)]. Sham and Hx rats were given either twice-daily s.c. injections of 0.9% saline solution (normal saline) for 7 days or s.c. implantation of a Silastic tubing (sham, Hx). A, Top, representative Western blot for hepatic CYP2C11 apoprotein. Bar, mean ± S.E.M. in ADU in which the value of sham was defined as 100% (n = 7–15). B, Top, representative Northern blot for steady-state mRNA of hepatic CYP2C11. Quantification of CYP2C11 bands from Northern blots was obtained by using a laser scanning densitometer, and the values are normalized to 28S RNA. Bar, mean ± S.E.M. ADU in which the value of sham was defined as 100% (n = 7–15). Mean values with different letters differ by P < .05.
0.9% saline solution, sham plus normal saline; 2) sham-Hx rats subcutaneously implanted a Silastic tubing (ST) of similar size to that of minipump, sham plus ST; (3) Hx rats receiving twice-daily s.c. injection of 0.9% saline solution (normal saline); Hx plus normal saline, Hx rats receiving twice-daily s.c. injection of 0.9% saline solution (normal saline); Hx rats receiving twice-daily s.c. injection of normal saline for 7 days. A, Top, representative Western blot for CYP2C11 apoprotein. B, Top, representative Northern blot for steady-state mRNA of hepatic CYP2C11. Quantification of CYP2C11 bands from Northern blot was obtained using a laser scanning densitometer, and the values are normalized to 28S RNA. Bar, mean ± S.E.M. ADU where the value from sham was defined as 100% (n = 5–10). Mean values bearing different letters differ by P < .05.

Fig. 3. Effects of Hx and anti-rGH serum treatment on rat body weight gains and serum IGF-I concentrations. Rat body weight gains (top) and serum IGF-I concentrations (bottom). Intact rats were either given twice-daily s.c. injection of 150 mg γ-globulin fraction of sheep anti-rGH serum per injection (anti-rGH) or same volume of vehicle (vehicle) for 11 days. Sham and Hx rats were given twice-daily s.c. injection of 0.9% saline solution 4 days after surgery for 7 days. Body weights were recorded and serum was collected. Values are mean ± S.E.M. of n = 10 to 11 per group. ***, P < .001 compared with respective controls.
in place of nonimmune sheep IgG was based on previous experiments demonstrating that Na₂HPO₄ produced the same results as nonimmune sheep IgG (Gardner and Flint, 1989). Rat body weights were determined daily throughout the experiment. The effectiveness of anti-rGH serum on GH neutralization was verified by monitoring body weight gains and measuring serum IGF-I concentrations. Rats were killed by decapitation after 11 days of anti-rGH serum or vehicle treatment, trunk blood was collected for hormonal assay and tissues were collected and stored at −70°C until microsomes and RNA were prepared as previously described (Badger et al., 1993).

Western blot analysis. Isolated microsomes were assayed by Western blotting to determine the level of CYP2C11 apoprotein following the previously described procedure (Badger et al., 1993). Briefly, 2.5 to 25 μg of microsomal protein from each animal was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Separated proteins were blotted onto nitrocellulose and probed with an specific mouse monoclonal antibody directed against rat CYP2C11 (Reik et al., 1987). ¹²⁵I-Labeled goat anti-mouse IgG was used as the secondary antibody for hepatic microsomes. Goat anti-mouse IgG conjugated with horseradish peroxidase was used as the secondary antibody in detecting renal CYP2C11 using enhanced chemiluminescence (ECL; Amersham Life Science, Arlington Heights, IL). Immunoquantification was accomplished by densitometric scanning of the radiographs.

Northern blot analysis. Isolation of total RNA, electrophoresis of 10 μg of total RNA, transfer onto Zeta Probe nylon membrane (BioRad, Hercules, CA) and hybridization with ³²P-labeled CYP2C11 oligonucleotides were conducted according to a previously reported method (Badger et al., 1993). Kidney poly(A)+ RNA was isolated from total RNA using the Poly-A-Tract mRNA Isolation System (Promega, Madison, WI) according to manufacturer’s instructions; 2 μg was used in the assay.

Preparation of Pit. Pituitaries of sexually mature Sprague-Dawley rats (200–500 g) were purchased from Pel-Freez (Rogers, AR). Pits were prepared by a modification of the methods of Campbell et al. (1978) and Skett and Young (Young, 1982). Briefly, rat pituitaries were homogenized with 15 strokes in 0.9% saline solution (25 pituitaries/ml of saline solution) using a hand-held homogenizer. The resulting homogenate was centrifuged at 28,000 × g at 4°C for 30 min. The resulting supernates were used as pituitary extract.

RIAs. The content of rat GH in Pit and LH concentrations were determined by RIA as previously described (Badger et al., 1991, 1982) using materials provided by the National Hormone and Pituitary Program and rGH RP-2 and rLH RP-2 as the standard, respectively. IGF-I concentrations were assessed in the acid/ethanol extract with a commercially available RIA kit (Nichols Institute Diagnostics, San Juan Capistrano, CA) using the manufacturer’s procedure. Serum T concentrations were assessed using a commercially available kit (Diagnostic Systems Laboratories, Webster, TX) according to the manufacturer’s procedure. All samples were assayed within the same respective RIA, and the intra-assay coefficients of variation were <4%.

Statistics. All data are expressed as mean ± S.E.M. for 5 to 11 individual rats/group. Comparisons of data between experimental groups for experiments 1 and 2 were made using one-way analysis of variance followed by the Student-Newman-Keuls method. The Student’s t test was used for data in experiment 3.

Results

Effects of Hx and hormonal replacement on body weight gains and serum IGF-I concentrations. To determine the completeness of Hx and the effectiveness of hor-
mone replacement, we measured rat body weight gains from the start (4 days after sham or Hx surgery) to the end of hormone replacement treatment (11 days after surgery) as well as serum IGF-I concentrations from sham and Hx rats (experiment 2). Sham-operated control rats gained an average of 49.9 g after 7 days of 0.9% saline solution treatment, whereas Hx rats gained essentially no weight during the same period (table 1). In parallel with the lack of body weight gain, serum IGF-I concentrations decreased 97% after Hx (P < .001) (table 1). Replacement of Hx rats with rGH or Pit restored the mean body weight gain to that of sham-operated rats. The effect of Pit on the mean body weight gain appeared to not require gonadal steroids because rats that were Hx and castrated responded to the extract by gaining weight at a rate nearly equal to controls. The mean body weight gain of hGH-treated Hx rats nearly attained rates of sham-operated controls, whereas Hx rats treated with T had mean body weight gain of 20% of sham controls (table 1). Similar results of the mean body weight gain were also observed in experiment 1 (data not shown).

**Effects of Hx and hormonal replacement on hepatic CYP2C11 apoprotein, steady-state mRNA levels.** In experiment 1, mean CYP2C11 apoprotein and mRNA levels of sham-Hx rats receiving either s.c. injection of 0.9% saline solution or s.c. implantation of Silastic tubing did not differ significantly, nor did Hx rats receiving s.c. injection of saline solution or Silastic tubing implantation (data not shown). Thus, data from these respective groups were combined and designated as sham or Hx, respectively. Pituitary hormone depletion of male rats by Hx reduced (P < .05) the mean hepatic CYP2C11 apoprotein level (fig. 1A). Replacement of Hx rats with twice-daily s.c. injection of either hGH or Pit prevented the decline in the mean CYP2C11 apoprotein level from that of sham-operated rats. The mean hepatic CYP2C11 apoprotein level was further reduced (P < .05) in Hx rats by continuous infusion of hGH. The CYP2C11 activity, as measured by 2α- and 16α-hydroxylation of T, paralleled the changes in apoprotein (data not shown). Steady-state levels of hepatic CYP2C11 mRNA in Hx and hormonal replacement rats paralleled the changes in hepatic CYP2C11 apoprotein levels (fig. 1B). The results from Hx and hGH replacement are consistent with previous reports (Janeczko et al., 1990; Kato et al., 1986; Morgan et al., 1985).

In experiment 2, pituitary hormone ablation of male rats by Hx resulted in decreased (P < .05) mean hepatic CYP2C11 apoprotein level (fig. 2A). Effects of hGH or Pit replacement on hepatic CYP2C11 apoprotein level were similar to those in experiment 1. Replacement of Hx rats with 133 μg rGH/ injection increased the mean CYP2C11 apoprotein level but failed to completely prevent the Hx-induced decrease, whereas pituitary extract completely blocked the CYP2C11 decline (fig. 2A). Importantly, the maintenance of hepatic CYP2C11 apoprotein level by Pit occurred in the presence or absence of the testes (fig. 2A). Treatment of Hx or Hx-plus-C rats with T (3 mg/kg/day) did not prevent loss of hepatic CYP2C11 apoprotein (fig. 2A). These results indicate, as previously reported (Janeczko et al., 1990; Kato et al., 1986; Morgan et al., 1985), that hepatic CYP2C11 is regulated by GH rather than by T or its metabolites originating from exogenous T. Hx of male rats also resulted in decreased (P < .05) mean hepatic CYP2C11 mRNA levels (fig. 2B), and the patterns of effects of hormonal replacement and castration plus replacement were similar to those of the apoprotein.

**Effects of anti-rGH serum on body weight gains, serum IGF-I concentrations and hepatic CYP2C11 apoprotein and mRNA levels.** To evaluate the effectiveness of sheep anti-rGH serum on neutralization of GH, rat body weight gains were determined from the beginning to the end of serum treatment and serum IGF-I concentrations were assessed at the time of death. Treatment with γ-globulin fraction of sheep anti-rGH serum for 11 days resulted in decreased (P < .05) mean body weight gain compared with vehicle control, and this was accompanied by decreased (P < .05) mean serum IGF-I concentrations (fig. 3). It should be noted that although Hx nearly eliminated body weight gains and drastically reduced serum IGF-I concentrations, anti-serum treatment was less effective. There were no differences in mean body weight gain and serum IGF-I concentrations between vehicle-treated control rats and sham-operated control rats (fig. 3). Neutralization of rGH by treatment of intact male rats with sheep anti-rGH serum resulted in decreased (P < .05) mean hepatic CYP2C11 apoprotein level (fig. 4A), which was accompanied by decreased (P < .05) mean CYP2C11 mRNA level (fig. 4B). The combined results from Hx rats and anti-rGH serum-treated rats strongly suggest an significant role for GH in the regulation of rat hepatic CYP2C11 at the level of mRNA and support previous work demonstrating a transcriptional regulation (Legraverend et al., 1992).

**Effects of Hx and hormonal replacement on renal CYP2C11 apoprotein and steady-state mRNA levels.** Western and Northern blot analyses from two experiments demonstrated the effectiveness of CYP2C11 apoprotein and mRNA in male rat kidney (figs. 5 and 6). Hx of male rats in experiments 1 and 2 resulted in decreased (P < .05) mean renal CYP2C11 apoprotein level (fig. 5, A and B), an effect very similar to that observed in liver. However, unlike in the liver, treatment of Hx rats with exogenous hGH or rGH did not prevent the decline of the mean renal CYP2C11 apoprotein level, whereas replacement with Pit did (fig. 5, A and B). In fact, the apoprotein level of pituitary extract-treated rats in experiment 1 exceeded (P < .05) the level of controls. The CYP2C11 activity, as measured by 2α- and 16α-hydroxylation of T, paralleled the changes in apoprotein (data not shown).

In experiment 2, T replacement was demonstrated to have the same effect as pituitary extract (fig. 5B), and the pituitary extract effect was dependent on the existence of the testes, as established by a failure of the pituitary extract to prevent the decline in mean CYP2C11 apoprotein level in castrated rats (fig. 5B). Furthermore, T was able to block the decline in renal CYP2C11 apoprotein levels in Hx and Hx-plus-castrated rats (fig. 5B). Similar results were observed with renal CYP2C11 mRNA levels (fig. 6), suggesting that renal CYP2C11 is regulated by gonadal steroids.

Based on calculations from our Western blot analysis, the amount of immunoreactive CYP2C11 apoprotein in kidney (using hepatic probes) is approximately one tenth of that in liver when expressed as the amount of CYP2C11 apoprotein/mg microsomal protein.

**Effects of anti-rGH serum on renal CYP2C11 apoprotein and mRNA levels and serum T and LH concentrations.** Although neutralization of rGH with anti-rGH serum
had the tendency to reduce the level of renal CYP2C11 apoprotein (fig. 7A), it failed to reach the statistical significance (P = 0.11). However, anti-GH serum treatment decreased the mean CYP2C11 mRNA level (P < .05) (fig. 7B). Based on the results of androgen replacement to castrated rats, the data in figure 7 suggested that serum T might be reduced in anti-serum-treated rats. Figure 8 demonstrates that although serum LH concentrations were not altered by the anti-serum treatment, serum T concentrations were reduced (P < .001). These results, along with those from Hx rat models, strongly suggest that gonadal steroids up-regulate the constitutive expression of renal CYP2C11, at least at the level of mRNA.

Discussion

We used the Hx rat as a model to evaluate hormonal regulation of CYP2C11 in liver and kidney. Hx of male rats led to decreased hepatic CYP2C11 apoprotein and mRNA levels. Twice-daily s.c. GH injections were used because previous studies of CYP2C11 regulation demonstrated that this mode of GH administration sufficiently approximated the pulsatile GH profile of male rats (high-amplitude GH pulses separated by nadirs of nearly undetectable GH) to restore CYP2C11 to control levels (Waxman et al., 1991). Similarly, continuous GH infusion was used to experimentally approximate the female rat serum GH profile, in which GH concentrations are always detectable. The twice-daily GH injections restored CYP2C11 apoprotein and mRNA levels of Hx rats to normal male levels and the GH infusion further reduced the CYP2C11 levels of Hx rats toward female values, which is consistent with previous reports (Janeczko et al., 1990; Kato et al., 1986; Morgan et al., 1985).

Although hGH can bind to both GH and prolactin receptors of liver and exhibits somatogenic and lactogenic activity (Norstedt and Palmiter, 1984; Postel-Vinay, 1976; Ranke et al., 1976), the effects of hGH on rat hepatic CYP2C11 and CYP2C12 apoprotein levels have been regarded to be mediated through a somatogenic receptor because ovine prolactin (an specific lactogen in the rat) had no effects on the levels of CYP2C11 and CYP2C12 apoprotein (MacGeoch et al., 1985; Morgan et al., 1985). Pit also restored hepatic CYP2C11 apoprotein and mRNA to levels equal to sham controls even when the amount of immunoreactive rGH in the extract was lower than that of purified hGH or rGH.

Taken together, data from our studies strongly support previous reports (Janeczko et al., 1990; Kamataki et al., 1985; Kato et al., 1986; Morgan et al., 1985), indicating that hepatic CYP2C11 is directly regulated by GH and not gonadal steroids. These data are (1) T replacement of Hx rat failed to restore hepatic CYP2C11 apoprotein and mRNA levels reduced by Hx and (2) the effectiveness of Pit to restore hepatic CYP2C11 apoprotein and mRNA levels was independent of testicular action because the extract was able to restore hepatic CYP2C11 apoprotein and mRNA levels in Hx-plus-castrated rats.

One disadvantage of the Hx model is the resultant multiple hormone deficiency that makes identification of specific GH...
effects difficult, even with hormonal replacement experiments. Thus, although the data discussed above from our laboratory and those from several other laboratories strongly implicate GH as the primary regulator of hepatic CYP2C11, other pituitary factors or interactions between factors could be involved. We chose therefore to use anti-rGH serum to neutralize circulating rGH and study the effects on the regulation of cytochrome P450 expression independent of other pituitary hormone deficiencies. To our knowledge, this is the first report to use this neutralization of GH to study CYP2C11 regulation. Neutralization of adult male rat GH for 11 days resulted in decreased body weight gain and serum IGF-I concentration, two indicators of effective GH neutralization by the anti-rGH serum treatment. Our results demonstrated that anti-rGH serum treatment decreased hepatic CYP2C11 apoprotein and mRNA levels. The effects of anti-rGH serum on CYP2C11 could not be attributed to neutralization of other pituitary hormones because specificity of this sheep anti-rGH serum has been demonstrated, with cross-reactivity being 0.5% for rat prolactin and rat LH, 0.4% for rat thyroid-stimulating hormone and 0.05% for rat adrenocorticotropic hormone (Madon et al., 1986). Thus, the findings that both Hx and anti-rGH serum treatment of male rats resulted in similar decreases of hepatic CYP2C11 apoprotein and mRNA levels provide additional support that GH stimulates hepatic CYP2C11 expression.

Although liver is the primary site of mammalian cytochrome P450s and drug metabolism, the P450-dependent monooxygenase system is present in many extrahepatic tissues, including kidney (Burke and Orrenius, 1979). Using hepatic probes, we estimate that rat kidney contains approximately one tenth the CYP2C11 apoprotein (per µg microsomal protein) contained in the liver. We demonstrated for the first time that renal CYP2C11 is regulated differently from the hepatic counterpart. In the studies conducted in Hx male rats, CYP2C11 apoprotein and mRNA levels decreased in kidney, which are similar to the effects in liver. Unlike the liver, however, Hx-induced decreases in renal CYP2C11 apoprotein and steady-state mRNA levels could not be prevented by GH replacement. Furthermore, gonadal steroids appear to stimulate renal CYP2C11 because T replacement of Hx or Hx-plus-castrated rats restored CYP2C11 apoprotein levels to at least as high as sham-operated controls, but pituitary extract replacement in castrated rats did not. Taken together, these results indicate that renal CYP2C11 is regulated by gonadal steroids and not by GH.

This conclusion was supported by the results of the anti-GH serum experiment. Anti-rGH serum treatment of adult male rats resulted in neutralization of rat GH but failed to significantly reduce renal CYP2C11 apoprotein level, which is in agreement with the results from Hx rat model suggesting that GH is not directly involved in the regulation of renal CYP2C11 in rats. There were, however, two unexpected observations made in that experiment. First, renal CYP2C11 mRNA levels were significantly suppressed, and second, the anti-rGH serum-treated rats had significantly reduced serum T levels. The reduction in serum T was unanticipated because, as mentioned above, this antiserum has been re-
peatedly tested for cross-reactivity with other pituitary hormones, including LH, and found to be highly specific for GH. Because LH was not decreased in the present study, the decline in serum T concentrations may be due to a direct or indirect effect of the antiserum on testicular production of T. Nevertheless, because GH and T replacement experiments demonstrated that T (and not GH) prevented renal CYP2C11 decline with Hx, we concluded that the reduction in serum T appeared to be responsible for the reduced renal CYP2C11 levels in rat treated with anti-rGH serum. Therefore, the results from both model systems (Hx and anti-rGH serum) strongly suggest that renal CYP2C11 is under the influence of gonadal steroids (probably androgens) and not by GH.

The existence of tissue-specific regulation of P450s between liver and kidney is not uncommon. CYP4A2, a lauric acid hydroxylase and a major cytochrome P450 in male rat kidney, is similar to a major renal cytochrome P450, P450 HK, in humans (Imaoka et al., 1990). The expression of CYP4A2 was reported to be directly regulated by T and GH in kidney at the levels of apoprotein and enzyme activity (Imaoka et al., 1992), although its expression is regulated mostly by GH in liver. It was also reported that treatment of Hx rats with T, not GH, had effects on renal CYP4A2 mRNA levels in rat (Sundseth and Waxman, 1992). Similarly, GH appeared to be involved in regulation of mouse hepatic CYP4A protein, whereas T was the key regulatory factor for renal CYP4A protein, and phenobarbital or dexamethasone administration affected the expression of CYP4A protein in liver but not in kidneys (Henderson et al., 1994). Furthermore, T has been implicated in the regulation of other renal P450s in mice. Henderson et al. (1990) reported the extensive sexual dimorphism in the expression of mouse kidney P450s and showed that T, not GH, mediates this effect. Testosterone has been implicated as the factor responsible for the much higher CYP2E1 activity in male mouse kidney than in females associated with metabolic activation of acetaminophen and acetaminophen-mediated toxicity in mouse kidney (Hu et al., 1993). Further studies indicated that the effects of T on the sexual dimorphic expression of mouse kidney P450s was mediated through androgen receptor (Henderson and Wolf, 1991).

In summary, rat liver and kidney contain CYP2C11 apoprotein and mRNA. Although GH regulates hepatic CYP2C11, gonadal steroids up-regulate CYP2C11 in rat kidney. Studies are under way to determine whether this indeed is an androgen-mediated response. We report here for the first time the use of anti-rGH serum to neutralize rat GH to study the effects of GH on the regulation of CYP2C11, an important enzyme in the phase I metabolism of endogenous compounds (i.e., steroids) and xenobiotics. Further studies are in progress in our laboratory to determine the molecular mechanisms of GH and steroid regulation of hepatic and extrahepatic CYP2C11.
Fig. 8. Effects of anti-GH serum on serum T and LH concentrations. Serum T concentrations (top) and serum LH concentrations after 11 days of anti-GH serum treatment (n = 10 to 11). ***, P < .001.

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