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THE MICROSOMAL MONOOXYGENASE SYSTEM OF REGENERATING LIVER

AN EXAMINATION OF THE ROLE OF ESTRADIOL IN THE DEMASCULINIZATION OF DRUG METABOLISM PRODUCED BY 2/3 PARTIAL HEPATECTOMY

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Abstract—Declines in total cytochrome P450 content and in monooxygenase activities associated with some male specific isozymes of cytochrome P450 have been reported in the rat following 2/3 partial hepatectomy (2/3 PH). In the present study, we examined the effects of 2/3 PH on hepatic microsomal monooxygenase activities towards testosterone, the alkoxyresorufins, *p*-nitrophenol and carbon tetrachloride in male rats. Levels of P450 apoproteins were determined by Western blot analysis. The effects of hepatectomy and sham operations on plasma growth hormone (GH) pulse profiles and the effects of a single acute dose of estradiol (E2) were studied to determine the role of these factors in 2/3 PH mediated changes in oxidative metabolism. 2/3 PH produced substantial decreases in testosterone hydroxylation at positions 16α, 2α and 7α, but only a small decrease in hydroxylation at position 6β. Reductions in CYP 2C11 (P450h) and CYP 2A1 (P450a) expression were observed with Western blot analysis down to 19 and 41% of control values, respectively, but insignificant effects were observed on expression of CYP 3A (P450p family) proteins recognized by a polyclonal antibody raised against rat CYP 3A2 (P450pcn2). In contrast, acute E2 treatment caused a 2-fold increase in expression of CYP 2A1 apoprotein and significantly decreased expression of CYP 2E1 (P450j) apoprotein and dependent monooxygenase activities, but had no significant effect on expression of CYP 2C11. Both sham operations and 2/3 PH caused a temporary decrease in plasma GH concentrations, but secretion returned towards normal 24–48 hr after both operations. These data suggest that some factor other than GH or E2 must be involved in the selective suppression of some P450 isozymes observed after 2/3 PH.

With the development of transplant procedures involving transfer of single liver lobes from living donors to recipients and subsequent liver regeneration in both, much interest has focused on the metabolic changes associated with regeneration. These changes in metabolism are important for the post-surgical medical management of these patients since patterns of drug metabolism are altered radically. In addition, the risk of immortalizing mutations produced by activation of procarcinogens by microsomal monooxygenase has heightened interest in the mechanisms regulating cytochrome P450 expression during the regenerative process [1, 2]. Reduction of monooxygenase activity during liver regeneration in the male rat was first reported by Von der Decken and Hutlin in 1960 [3]. In the last few years, a number of studies have demonstrated specific declines lasting more than 1 week post-operation in the male specific P450 isozyme CYP 2C11 (P450h) and in the glucocorticoid inducible CYP 3A1 (P450pl) [4, 5]. A number of possible explanations have been advanced for the apparent demasculinization of drug metabolism associated with regeneration. The first and most obvious one relates to the significant stress associated with 2/3 partial hepatectomy (2/3 PH§). Another possible explanation is a feminization of growth hormone (GH) pulse profiles after 2/3 PH. This explanation is appealing because GH has been shown to be responsible for the maintenance of male specific P450 isozymes [6, 7]. Significant perturbations in plasma GH pulse patterns could alter the levels and activities of these isozymes. A third explanation involves the reported acute 8-fold rise in circulating estradiol (E2) levels which peak at 6 hr after 2/3 PH. It has been suggested that this E2 spike may be involved in both the regeneration process itself and in the regeneration-associated demasculinization of drug metabolism [4, 8]. In the current study, the

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§ Abbreviations: 2/3 PH, 2/3 partial hepatectomy; GH, growth hormone; E2, estradiol; and HMO, hepatic monooxygenase system.

effects of 2/3 PH on the hepatic monooxygenase system (HMO) were examined 48 hr post-hepatectomy by studying the metabolism of P450 isozyme specific substrates. To mimic the previously reported rise in E2 after 2/3 PH, the acute effects of a single injection of estradiol were studied on control and sham-operated animals and, in addition, the effects of sham operation and 2/3 PH were determined on plasma GH profiles.

MATERIALS AND METHODS

Virus-free adult male Sprague–Dawley rats were obtained from Taconic Breeders Inc. (New York, NY). LHP-KDF normal phase, silica gel HPTLC plates were supplied by the Whatman Instrumental Co. (Maidstone, Kent, U.K.). Ethoxyresorufin, pentoxyresorufin and resorufin were purchased from the Pierce Chemical Co. (Rockford, IL). [^{14}C]-Testosterone (50–60 mCi/mmol) was purchased from New England Nuclear (Boston, MA) and ^{125}I-goat anti-rabbit IgG (2–15 μCi/μg) was supplied by ICN Biomedicals, Inc. (Costa Mesa, CA). Testosterone, thiobarbituric acid and NADPH were from the Sigma Chemical Co. (St. Louis, MO). 16α-Hydroxytestosterone, 7α-hydroxytestosterone and 6β-hydroxytestosterone were obtained from Steraloids Inc. (Wilton, NH). Other testosterone metabolites were obtained from the MRC Steroid Reference Collection (St. Mary's Hospital, London, U.K.). Carbon tetrachloride was from the Aldrich Chemical Co. (Milwaukee, WI) and *p*-nitrophenol from the Fisher Scientific Co. (Springfield, NJ). Rabbit polyclonal antibodies against CYP 2E1 and mouse monoclonal antibodies against CYP 2C11 were prepared as described previously [9, 10]. Rabbit polyclonal antibodies directed against CYP 2A1 (rat P450a) and an isozyme identified as CYP 3A2 (rat P450pcn2) [11] were the gift of Dr. Anders Astrom, Karolinska Institute (Huddinge, Sweden).

Three groups (N = 5) of male Sprague–Dawley rats (275 g) were anesthetized with isoflurane. Two groups (Sham and Sham + E2) were subjected to sham operations during which the abdomen was opened, the liver manipulated and the excision sutured. A third group had approximately 66% of the liver mass removed during partial hepatectomy (Regen) as described by Higgins and Anderson [12]. Six hours later, E2 (4 μg/100 g body wt in corn oil) was injected i.p. into the Sham + E2 group and into another group (N = 5) of unoperated animals (NC + E2). We have reported previously that this dose achieved serum E2 concentrations between 776 and 903 pg/mL, a concentration reasoned to be sufficient to produce estrogen-induced effects [13]. All rats were killed 48 hr following either sham operations or 2/3 PH.

Hepatic microsomes were prepared [14] from livers removed at necropsy and during partial hepatectomy. Levels of monooxygenase components, total cytochrome P450, cytochrome b_5 and P450 reductase were assayed spectrophotometrically by the methods of Omura and Sato [15] and Dignam and Strobel [16]. Monooxygenase activities were measured as follows: CYP 2E1 (P450j)-dependent activity was determined indirectly by measurement of carbon tetrachloride dependent lipid peroxidation using the method of Johansson and Ingelman-Sundberg [17], and directly by measurement of the hydroxylation of *p*-nitrophenol to *p*-nitrocatechol [18]. CYP 2B1 (P450b) and CYP 1A1 (P450c)-dependent activities were determined by fluorometric measurement of the dealkylation of pentoxy- and ethoxyresorufin [19, 20]. Testosterone metabolism was assessed by the methods of Waxman *et al.* [21]. Testosterone and its metabolites were separated by HPTLC on Whatman normal phase, silica gel, LHP-

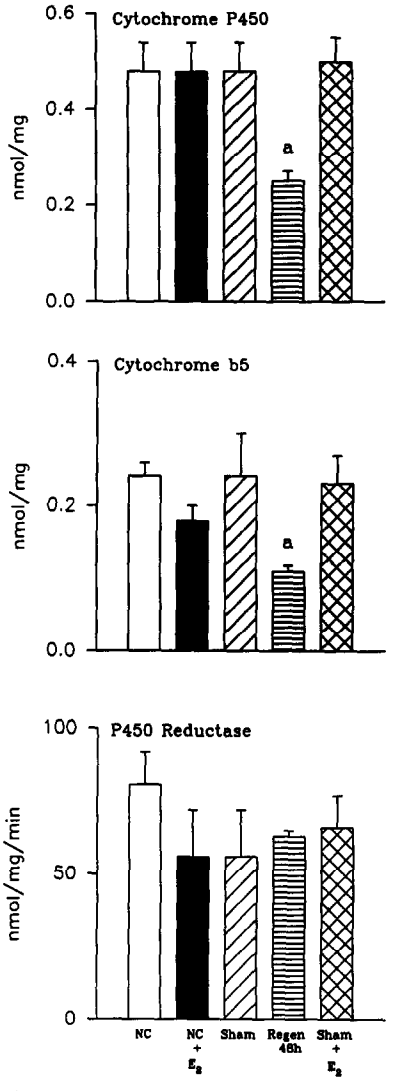

Fig. 1. Effects of regeneration and E2 on monooxygenase components. Top panel: spectrally determined content of hepatic cytochrome P450. Middle panel: spectrally determined content of hepatic cytochrome b_5. Bottom panel: hepatic cytochrome P450 reductase activity determined by the reduction of cytochrome *c*. Values are means ± SEM for N = 5 per group. Key: (a) $P \ll 0.005$. Treatment groups: NC, naive control rats; NC + E2, naive control rats treated with E2; Sham, sham-operated rats; Regen, rats with 2/3 partial hepatectomies; and Sham + E2, sham-operated rats treated with E2.

Table 1. Effects of acute E2 treatment and liver regeneration on monooxygenase activities

Treatment	EROD	PROD	pNP (pmol/mg/min)	CCl_4*
NC	140 ± 21	28 ± 4	120 ± 10	52 ± 13
Sham	87 ± 18†	16 ± 4†	125 ± 18	52 ± 6
NC + E2	106 ± 17	19 ± 3	90 ± 10‡	28 ± 5§
Sham + E2	118 ± 11	18 ± 5	99 ± 13	14 ± 7‖
Regen 48 hr	95 ± 12	17 ± 2	130 ± 30	52 ± 6

Values are means ± SEM of N = 5 per group. See Fig. 1 for definitions of treatments. Abbreviations: EROD, ethoxyresorufin *O*-deethylase; PROD, pentoxyresorufin *O*-dependentylase; and pNP, *p*-nitrophenol hydroxylase.

* CCl_4-dependent lipid peroxidation was measured as the rate of formation of thiobarbituric acid reactive products.

† $P \leq 0.05$, NC vs Sham.

‡ $P \leq 0.05$, NC vs NC + E2.

§ $P \leq 0.01$, NC vs NC + E2.

‖ $P \leq 0.005$, Sham vs Sham + E2.

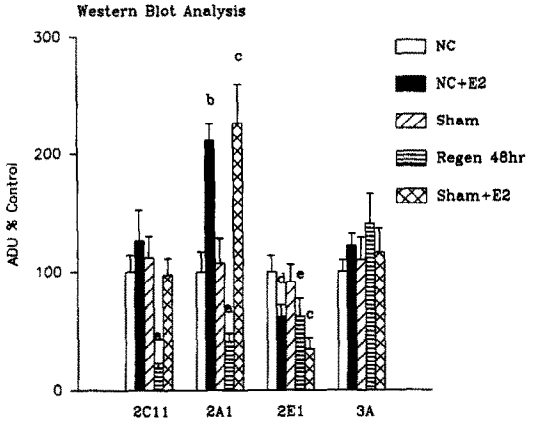

Fig. 2. Immunoquantitation of expression of cytochrome P450 isozymes CYP 2C11 (P450h), CYP 2A1 (P450a), CYP 2E1 (P450j) and CYP 3A (P450p family) in hepatic microsomes following 2/3 PH or acute estradiol treatment. Data are means ± SEM for five individual animals in each treatment group compared to naive controls. Naive controls equal 100% based on densitometric scanning of autoradiographs generated by Western blot analysis using 5 μg microsomal protein/well. ADU = autoradiographic density units. See Fig. 1 legend for definitions of treatments. Key: (a) $P \leq 0.005$, Sham vs Regen; (b) $P \leq 0.005$, NC vs NC + E2; (c) $P \leq 0.005$, Sham vs Sham + E2; (d) $P \leq 0.01$, NC vs NC + E2; and (e) $P \leq 0.05$, Sham vs Regen.

KDF HPTLC plates and run twice in the same dimension in a solvent system of dichloromethane:acetone (4:1). The HPTLC plates were subjected to autoradiography overnight, and testosterone turnover was quantitated by densitometric scanning of the autoradiographs using an EC910 densitometer. Preliminary experiments demonstrated that densitometric scanning yields results equivalent to scrapping the HPTLC plates and counting the radioactivity of each band. Products were identified by co-migration on the HPTLC plates with metabolite standards and by GC–MS.

Total protein concentration was determined using Bio-Rad protein assay reagent in accordance with manufacturer's instructions. Levels of apoprotein of P450 isozymes CYP 3A (P450p family), CYP 2A1 (P450a), CYP 2C11 (P450h) and CYP 2E1 (P450j) were followed by Western blot analysis as described by Ronis *et al.* [22] using rabbit polyclonal antibodies against CYP 2E1, CYP 2A1 and CYP 3A2 and a mouse monoclonal antibody directed against CYP 2C11 and ^{125}I-labeled goat anti-rabbit and anti-mouse IgG as the secondary antibodies. Immunoquantitation of the Western blots was accomplished by densitometry scanning of the resulting autoradiographs using an EC 910 densitometer.

The effects of surgery on plasma growth hormone profiles were examined using methods reported previously [23]. At the time of either the sham operation or 2/3 HP, an intrajugular cannula was implanted into Sham or 2/3 PH rats and was tunnelled subcutaneously to the dorsal aspect of the neck where it exited. Blood was collected every 10 min to determine plasma GH concentrations by radioimmunoassay. Longer periods of blood collection were contempleted, but to reduce excess blood removal a shorter period of 2 hr was selected. In addition, a group of naive control animals were implanted with an intrajugular cannula and allowed 14 days to recover from surgery before blood samples were collected every 10 min for 6 hr as described previously [23].

One-way analysis of variance followed by Newman–Keuls multiple range test was used to determine treatment effects.

RESULTS

Effects of sham operation on HMO. Forty-eight hours after sham operations, no significant effects were observed on levels of monooxygenase components—cytochrome P450, cytochrome b_5 or cytochrome P450 reductase (Fig. 1). A decline occurred in both ethoxy- and pentoxyresorufin *O*-dealkylase activities over those of naive control animals (Table 1). No significant effects were

observed on the levels of CYP 2C11, CYP 2A1, CYP 2E1 or CYP 3A apoproteins in Western blot analysis (Fig. 2).

Effect of regeneration on HMO. No significant effects were observed on cytochrome P450 reductase activity compared to sham-operated controls. However, 48 hr post-hepatectomy, total cytochrome P450 and cytochrome b_5 contents were less than 50% of sham-operated values (Fig. 1). Ethoxy- and pentoxyresorufin *O*-dealkylase activities appeared unaffected (Table 1). Although no effects were observed on CYP 2E1-dependent *p*-nitrophenol hydroxylase or carbon tetrachloride-dependent lipid peroxidation (Table 1), a small but significant decline in CYP 2E1 apoprotein was observed in Western blot analysis compared to sham-operated controls (Fig. 2). The metabolism of testosterone declined significantly after 2/3 PH, reflecting specific decreases in hydroxylation at positions 2α, 6β, 7α and 16α (Fig. 3). 16α- and 2α-Hydroxylation of testosterone are catalyzed predominantly by the male specific CYP 2C11, while hydroxylation at position 7α- is catalyzed by the female predominant CYP 2A1.

The decrease in metabolism of testosterone coincided with a decline in CYP 2C11 apoprotein to 19% of control levels and in CYP 2A1 apoprotein to 41% of control levels in Western blot analysis (Figs 2 and 4). The 20% decline ($P \leq 0.05$) in hydroxylation of testosterone at position 6β (Fig. 3) was not reflected in expression of CYP 3A apoproteins recognized in Western blot analysis using an antibody directed against CYP 3A2 (Fig. 2). Hydroxylation at the 6β position of testosterone is associated with P450 isozymes of gene family 3A.

Effects of acute E2 on HMO. Acute E2 given to either naive controls or sham-operated animals caused no significant declines in any monooxygenase component (Fig. 1). Declines in *p*-nitrophenol hydroxylase and carbon tetrachloride-dependent lipid peroxidation which are associated with CYP 2E1 were observed (Table 1) and a more marked decline was observed in CYP 2E1 apoprotein in Western blot analysis (Fig. 2). Although CYP 2A1 apoprotein levels doubled (Fig. 2), no significant effects were observed on the rate of testosterone hydroxylation at position 7α (Fig. 3).

Effect of sham operations and 2/3 PH on plasma GH profiles. Figure 5 illustrates the plasma GH concentrations over a 6-hr period in two representative control male rats that *had not* been subjected to either sham or 2/3 PH. Two large episodes of GH secretion occurred during this time, reaching plasma concentrations exceeding the 35 ng/mL maximum for the radioimmunoassay. Figure 6 depicts plasma GH profiles following sham operations and 2/3 PH. For comparative purposes, the first 2 hr of plasma GH concentrations from the control rats presented in Fig. 5 are shown in the upper two panels, along with data from representative Sham and 2/3 HP rats immediately following surgery (middle panels) or 48 hr following surgery (lower panels). As can be seen, the plasma GH concentrations are very low immediately following surgery in both Sham and 2/3 PH rats, with the mean plasma GH concentrations of all rats in both groups being less than 8 ng/mL. However, by 48 hr following either operation, plasma GH concentrations were much greater than 10 ng/mL and pulsatile secretion was evident. These data demonstrate that transient declines in the plasma GH concentrations occur immediately after surgical stress and begin to return towards control levels within the first 48 hr.

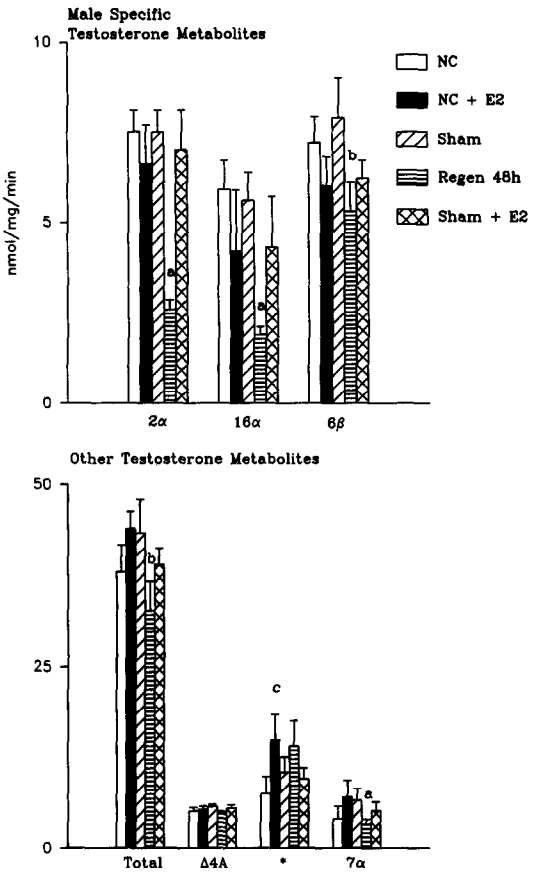

Fig. 3. Metabolism of testosterone in hepatic microsomes during liver regeneration or after E2 treatment. Data are means ± SEM for N = 5 per group. See Fig. 1 legend for definitions of treatments. Key: (a) $P \leq 0.005$, Sham vs Regen; (b) $P \leq 0.05$, Sham vs Regen; and (c) $P \leq 0.05$, NC vs NC + E2. Metabolites were identified by co-migration of cold standards on HPTLC and HPLC and by GC-MS. Total = total testosterone turnover; Δ4A = androstenedione; * = 5α-androstan-3$\alpha(\beta)$,17β-diol; 7α = 7α-hydroxytestosterone; 2α = 2α-hydroxytestosterone; 16α = 16α-hydroxytestosterone; and 6β = 6β-hydroxy-testosterone.

DISCUSSION

Short-term surgical effects on HMO. Small, transient surgical effects have been reported previously on levels of a number of P450 isozymes including CYP 2C11 and CYP 3A2 [24–26]. However, the time course and degree of these effects were highly variable. This may be related to the transient decrease in GH secretion reported here and also to

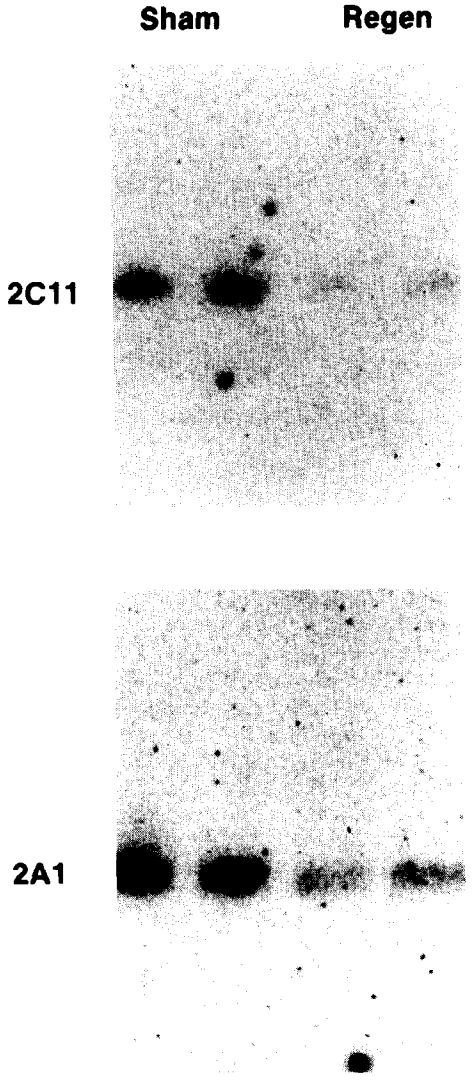

Fig. 4. Western blot analysis demonstrating the effect after 48 hr of 2/3 PH on hepatic microsomal expression of CYP 2C11 (P450h) and CYP 2A1 (P450a). Samples represent 5 μg of protein from pooled microsomes of five animals in each group assayed in duplicate. See Fig. 1 legend for treatment definitions.

short-term hormonal changes in response to stress [26]. Declines in alkoxyresorufin O-dealkylation were the only significant surgical effects observed in the present study.

Effects of 2/3 PH on HMO. Some P450 isozymes appear to be unaffected during the regenerative response. No effects of regeneration were observed on CYP 2B1-dependent pentoxyresorufin *O*-depentylase or CYP 1A1-dependent ethoxyresorufin *O*-deethylase activities. The selective decline in CYP 2C11 in liver microsomes after 2/3 HP is consistent with data from previous studies [4, 5]. Declines in CYP 2A1 expression have not been reported previously.

Only small decreases in CYP 3A-dependent activities (erthyromycin *N*-demethylase and androstenedione 6β-hydroxylase) have been reported in regeneration [4, 5]. However, using an antibody against a rabbit CYP 3A orthologue (rabbit P4503c, CYP 3A6) and a cDNA probe against *CYP 3A1* (450pcn1), it has been reported that almost complete suppression of CYP 3A1 occurs for over a week after 2/3 PH [5]. In the current study, at 48 hr after surgery a 20% decrease was observed in metabolism of testosterone at position 6β, while a small increase occurred in CYP 3A apoprotein expression in livers undergoing regeneration for 48 hr compared to sham controls when measured with a polyclonal antibody raised against CYP 3A2 (Fig. 2). The CYP 3A family is associated with 6β-hydroxylase and consists of at least four closely related isozymes [27, 28], two of which, CYP 3A1 (450pcn1) and CYP 3A2 (P450pcn2), have been cloned from the rat [29]. The apparent lack of agreement between the current report and previous data [5] may reflect separate mechanisms of regulation for the members of P450 gene family 3A. Although CYP 3A1 is suppressed by 2/3 PH, the major male specific CYP 3A form, 3A2 may be unaffected. This is supported by our Western analysis data using an antibody raised against CYP 3A2. More studies are required to further characterize and resolve the differential regulation of CYP 3A family members identified by different laboratories. Furthermore, since CYP 2C11 has been reported to have a small amount of testosterone 6β-OHase acitivity in addition to 2α- and 16α-OHase activity, it is possible that the small decrease in this activity observed in our studies reflects this suppression.

Effects of acute E2 on HMO. It has been reported that chronic treatment of male rats with E2 results in a profound suppression of testosterone 16α-hydroxylase activity [8] and that declines in hepatic 16α- and 6β-hydroxylase activities occur on intrapituitary or intrahypothalamic injection of E2 [30]. It is possible that there are both direct, chronic effects of E2 at the level of the liver, and indirect effects via changes in pituitary GH secretion. However, in the present study where E2 was administered acutely to mimic the previously reported rise in estrogens after 2/3 PH, no significant effects were observed on testosterone metabolism at positions 2α, 16α or 6β, nor were any significant effects observed on levels of CYP 2C11 or CYP 3A apoproteins in Western blot analysis. In contrast, it appears that acute E2 has effects on expression of P450 isozymes CYP 2A1 and CYP 2E1. CYP 2A1 was elevated 2-fold. It may be that circulating estrogens are involved in the maintenance of the higher levels of CYP 2A1 that are observed in female rats. *p*-Nitrophenol hydroxylase activity was suppressed by E2 to a smaller extent than the CYP 2E1 apoprotein. It may be that in E2-treated animals, this activity is catalyzed to some extent by P-450 isozymes other than CYP 2E1.

Possible mechanisms of cytochrome P450 suppression following 2/3 PH. Three hypotheses have been proposed for the mechanism of the selective suppression of CYP 2C11 in regenerating liver: (1) surgical stress; (2) a feminization of GH secretion from the anterior pituitary; and (3) a direct result

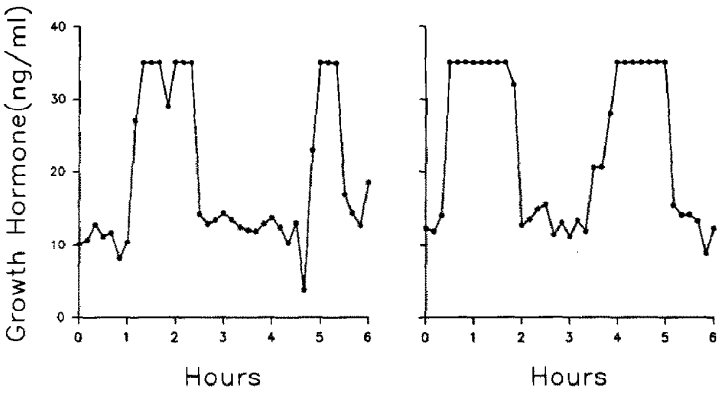

Fig. 5. Plasma GH profile of naive control rats. Data represent plasma GH concentrations from samples collected every 10 min for 6 hr.

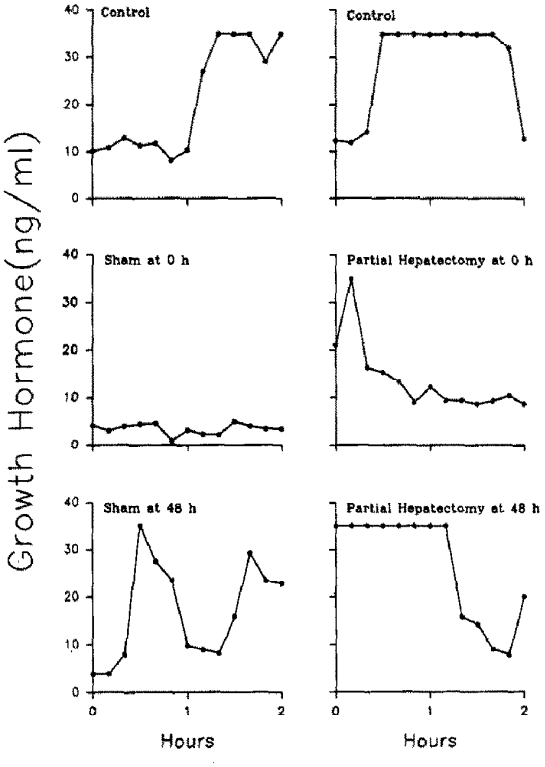

Fig. 6. Plasma growth hormone pulse profiles immediately following sham operation or 2/3 PH and 48 hr later. The data points represent GH concentrations of plasma collected every 10 min. Upper two panels: representative control male rats. Middle two panels: representative rats immediately following sham operation (Sham at 0 hr) or 2/3 HP (partial hepatectomy a 0 hr). Lower two panels: same rats as in middle panels after 48 hr.

of the rise in circulating estrogens seen after 2/3 PH. From the above results it appears that suppression of CYP 2C11 by surgical stress is unlikely. No significant sham effects were detectable on testosterone 2α- and 16α-hydroxylation or on CYP 2C11 apoprotein expression after 48 hr; whereas at the same time point an 80% reduction was observed after 2/3 PH. The second hypothesis also appears improbable. Transient alterations in GH secretion were observed in both sham-operated and 2/3 PH animals. If a feminization in GH pulse profile were responsible for the suppression of CYP 2C11, it would be expected that CYP 3A2 and its associated testosterone 6β-hydroxylase activity would also be substantially suppressed, since continuous GH administration has been shown to suppress CYP 3A2 expression [31]. However, after hypophysectomy where GH and other hormones from the anterior pituitary are eliminated, CYP 2C11 is suppressed, while CYP 3A2 is slightly elevated in a fashion similar to that observed in the present study [31].

With regard to the third hypothesis involving a direct effect of E2, Liddle *et al.* [4, 8] have described a transient 8-fold rise in circulating estrogens after 2/3 PH and suggest that it may play a role in CYP 2C11 suppression. The data presented above suggest that acute estradiol treatment which mimics the effect of 2/3 PH has no significant effects on either CYP 2C11 apoprotein expression or on its associated testosterone 2α- and 16α-hydroxylase activity. It is possible that local alterations in androgen and estrogen metabolism, in particular the suppression of estradiol 2α- and 16α-hydroxylation, may result in increased occupation of hepatic estrogen receptors in partially hepatectomized animals. That condition would be difficult to experimentally duplicate even using supraphysiologic concenrations of estradiol. However, our data suggest that some factor other than surgical stress, GH secretion or plasma E2 is involved in the suppression of CYP 2C11 regenerating liver. It has been suggested that hepatocyte growth factor (HGF), epidermal growth factor (EGF) and other peptides may be involved in the regenerative process [32, 33], and it is possible that one of these may affect CYP 2C11 expression. However, it is as yet unclear what role, if any, the alterations in the monooxygenase system play in liver regeneration and why only certain P450 isozymes are suppressed.

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REFERENCES

1. Bismuth H and Houssein D, Reduced size orthotopic liver graft in hepatic transplantation in children. *Surgery* **95**: 367–370, 1984.
2. Diehl AM, Thorgeirsson SS, Bisgaad G and Steer CJ, Ethanol interferes with regeneration associated changes in biotransforming enzymes. *Hepatology* **10**: 320, 1989.
3. Von der Decken A and Hutlin T, The enzymic composition of rat liver microsomes during liver regeneration. *Exp Cell Res* **19**: 591–604, 1960.
4. Liddle C, Murray M and Farrell GC, Effect of liver regeneration on hepatic cytochrome P-450 isozymes and serum sex steroids in the male rat. *Gastroenterology* **96**: 864–872, 1989.
5. Jean Marie I, Dalet C, Blanchard J-M, Astre C, Szawlowski A, Saint Aubert B, Joyeux H and Maurel P, Inhibition of cytochrome P-450p (P450IIIA1) gene expression during liver regeneration from two-thirds hepatectomy in the rat. *Biochem Pharmacol* **37**: 3515–3521, 1988.
6. Morgan ET, MacGeogh C and Gustafsson J-A, Hormonal and developmental regulation of the expression of the hepatic microsomal steroid 16α-hydroxylase cytochrome P-450 apoprotein in the rat. *J Biol Chem* **260**: 11895–11898, 1985.
7. Waxman DJ, Hepatic enzymes of steroid metabolism. Regulation of growth hormone secretory patterns. In: *Cytochrome P450 Biochemistry and Biophysics* (Ed. Schuster I), pp. 464–471. Taylor & Francis, London, 1989.
8. Liddle C, Hollands M, Farrell GC and Little JM, Serum estrogen and testosterone concentrations following partial liver resection in man. *Gastroenterology* **96**: A622, 1989.
9. Johansson I, Eckstrom G, Scholte B, Puzycki D, Jornvall H and Ingelman-Sundberg M, Ethanol-, fasting- and acetone-inducible cytochromes P-450 in rat liver: Regulation and characteristics of enzymes belonging to the IIB and IIE gene subfamilies. *Biochemistry* **27**: 1925–1934, 1988.
10. Reik LM, Maines SL, Ryan DE, Levin W, Bandiera S and Thomas PE, A simple nonchromatographic purification procedure for monoclonal antibodies: Isolation of monoclonal antibodies against cytochrome P450 isozymes. *J Immunol Methods* **100**: 123–130, 1987.
11. Åström A and DePierre J, Metabolism of 2-acetylaminofluorene by eight different forms of cytochrome P-450 isolated from rat liver. *Carcinogenesis* **6**: 113–120, 1985.
12. Higgins GM and Anderson RM, Experimental pathology of the liver. 1. Restoration of the liver of the white rat following partial surgical removal. *Arch Pathol* **12**: 186–202, 1931.
13. Jeffery JR, MacLaughlin DT, Badger TM, Miller DC and Martuza RL, Hormonal modulation of Schwann cell tumors. *Ann NY Acad Sci* **486**: 371–383, 1986.
14. Chipman JK, Kuruckgy N and Walker CH, Comparative metabolism of a dieldrin analogue: Hepatic microsomal systems as models for metabolism in the whole animal. *Biochem Pharmacol* **28**: 64–70, 1978.
15. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification, and properties. *J Biol Chem* **239**: 2379–2385, 1964.
16. Dignam JD and Strobel HW, Preparation of homogeneous NADPH-cytochrome P-450 reductase from rat liver. *Biochem Biophys Res Commun* **63**: 848–852, 1975.
17. Johansson I and Ingelman-Sundberg M, Carbon tetrachloride-induced lipid peroxidation dependent on an ethanol-inducible form of rabbit liver microsomal cytochrome P-450. *FEBS Lett* **183**: 265–269, 1985.
18. Koop D, Hydroxylation of *p*-nitrophenol by rabbit ethanol-inducible cytochrome P-450 isozyme 3a. *Mol Pharmacol* **29**: 399–404, 1986.
19. Burke MD and Mayer RT, Ethoxyresorufin: Direct fluorimetric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. *Drug Metab Dispos* **2**: 583–588, 1974.
20. Lubet RA, Mayer RT, Cameron JW, Nims RW, Burke MD, Wolff T and Guengerich FP, Dealkylation of pentoxyresorufin: A rapid and sensitive assay for measuring induction of cytochromes P-450 by phenobarbital and other xenobiotics in the rat. *Arch Biochem Biophys* **238**: 43–48, 1985.
21. Waxman DJ, Ko A and Walsh C, Regioselectivity and stereoselectivity of androgen hydroxylations catalyzed by cytochrome P-450 isozymes purified from phenobarbital-induced rat liver. *J Biol Chem* **258**: 11937–11947, 1983.
22. Ronis MJJ, Hansson T, Borlakoglu J and Walker CH, Cytochromes P-450 of sea birds: Cross-reactivity studies with purified rat cytochromes. *Xenobiotica* **19**: 1167–1173, 1989.
23. Badger TM, Millard W, McCormick GF, Bowers CY and Martin JB, Effects of growth hormone releasing peptides on GH secretion in perfused pituitary cells of adult male rats. *Endocrinology* **115**: 1432–1439, 1984.
24. Ronis MJJ, Lumpkin CK, Ingelman-Sundberg M and Badger TM, The effect of short term ethanol on rat hepatic microsomal monooxygenase in two systems utilizing total enteral nutrition. *Alcohol Clin Exp Res* **15**: 693–699, 1991.
25. Babany G, Descatoire V, Corbic M, Gendre S, Degott C, Larrey D, Letteron P, Wandscheer J-C, Funck-Brentano C and Pessayre D, Regulation of renal cytochrome P-450. Effect of two-thirds hepatectomy, cholestasis, biliary cirrhosis and post-necrotic cirrhosis on hepatic and renal microsomal enzymes. *Biochem Pharmacol* **34**: 311–320, 1985.
26. Leakey JEA, McMillan JM, Bazare JJ, Harmon JR, Cunny HC and Arlotto MP, Regulation of rat liver cytochrome P-450 IIIA isozymes. *Toxicologist* **10**: 127, 1990.
27. Graves PE, Kaminsky LS and Halpert J, Evidence for functional and structural multiplicity of pregnenolone-16α-carbonitrile-inducible cytochrome P-450 isozymes in rat liver microsomes. *Biochemistry* **26**: 3887–3894, 1987.
28. Gonzalez FJ, Song B-J and Hardwick JP, Pregnenolone 16α-carbonitrile-inducible P-450 gene family: Gene conversion and differential regulation. *Mol Cell Biol* **6**: 2969–2976, 1986.
29. Gonzalez FJ, The molecular biology of cytochromes P-450. *Pharmacol Rev* **40**: 243–289, 1989.
30. Mode A and Norstedt G, Effects of gonadal steroid hormones on the hypothalamic-pituitary-liver axis in the control of sex differences in hepatic steroid metabolism in the rat. *J Endocrinol* **95**: 181–187, 1982.
31. Waxman DJ, Ram PA, Notani G, LeBlanc G, Alberta JA, Morrissey JJ and Sundseth S, Pituitary regulation of the male-specific steroid 6β-hydroxylase P-450 2a (gene product IIIA2) in adult rat liver. Suppressive influence of growth hormone and thyroxine acting at a pretranslational level. *Mol Endocrinol* **4**: 443–454, 1990.
32. Nakamura T, Nishizawa T, Hagiya M, Seki T, Shimshonishi M, Sigimura A, Tashiro K and Shimizu S, Molecular cloning and expression of human hepatocyte growth factor. *Nature* **342**: 440–443, 1989.
33. Marti U, Burwen SJ and Jones AL, Biological effects of epidermal growth factor, with emphasis on the gastrointestinal tract and liver. An update. *Hepatology* **9**: 126–132, 1989.