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Interferon Down Regulates the Male-Specific Cytochrome P450IIIA2 in Rat Liver

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SUMMARY

The aim of this study was to clarify the mechanism by which cytochrome P450 (P450)-mediated catalytic activity is decreased following interferon (IFN) administration. Microsomal steroid hydroxylation was assessed to test the hypothesis that IFN selectively decreases the activities of individual P450 isozymes in male rats. Thus, recombinant rat IFNγ (r-rat IFNγ) treatment produced 40% and 17% reductions in androst-4-ene-3,17-dione (androstenedione) 6β- and 16β-hydroxylation, respectively. Androstenedione 16α- and 7α-hydroxylation were unaltered following r-rat IFNγ treatment. Similar changes in the androstenedione hydroxylation pathways were observed following administration of naturally derived rat IFNα/β. Microsomal levels of P450IIIA2, the male-specific constitutive steroid 6β-hydroxylase, were lower after administration of r-rat IFNγ (42% of control fractions). Furthermore, hepatic P450IIIA2 mRNA was found to be decreased to a similar extent by r-rat IFNγ. These findings suggest that IFN selectively decreases the content of this isozyme by a mechanism involving altered mRNA regulation. Sex steroids were unlikely to have mediated the decrease in P450IIIA2 levels since serum estradiol and testosterone levels were unchanged by r-rat IFNγ. In order to determine whether IFN alters the expression of P450IIIA1, a steroid-inducible member of the P450IIIA gene subfamily which is not expressed in untreated rat liver, adult female rats (which lack P450IIIA2) were coadministered pregnenolone 16α-carbonitrile and r-rat IFNγ. However, IFN failed to impair the induction of androstenedione 6β-hydroxylation produced by pregnenolone 16α-carbonitrile. These findings suggest that although IFN decreases the expression of P450IIIA2, it may not down regulate the expression of other steroid-inducible P450IIIA proteins. In view of the existence of human P450IIIA orthologs which catalyze the metabolism of several important therapeutic agents, the findings of this study may help predict possible drug interactions in patients receiving IFN.

In humans, administration of IFN has been shown to reduce the clearance of both antipyrine and theophylline (1, 2). This is probably due to an IFN-mediated impairment of hepatic oxidative drug metabolism, since both drugs are metabolized by microsomal P450 (3, 4). Moreover, IFN and IFN inducers have been shown to reduce the level and catalytic activity of hepatic P450 in experimental animals (5–7). We recently reported that the rat was a useful model for the study of IFN-mediated inhibition of drug metabolism following the demonstration that autologous, but not heterologous, IFN reduced theophylline clearance and P450 levels (8). However, an outstanding question was whether IFN decreased all P450 isozymes or whether the effect was specific for certain forms of the cytochrome. Furthermore, there are conflicting data as to whether the reduction of P450 produced by IFN is caused by down regulation of the P450 apoprotein or involves other mechanisms (9, 10). The purpose of this study was to resolve some of these questions.

We first determined the optimal dose and timing of IFN administration that resulted in maximal suppression of P450 levels and mixed-function oxidase activities in male rats. In subsequent experiments, the regio- and stereospecific hydroxylation of androstenedione was used to examine the activity of individual P450 isozymes. It is now clear that the formation of the 16α-, 16β-, 6β-, and 7α-hydroxyandrostenedione metabolites are catalyzed by the P450 isozymes [following the nomenclature of Nebert *et al.* (11)] P450IIC11, P450IIB1 and P450IIB2, P450IIIA, and P450IIA1 and P450IIA2, respectively (12–14). The studies reported here showed that rat IFN selectively reduced the activity of the P450IIIA2-dependent androstenedione 6β-hydroxylase pathway in male rats. Complementary experiments revealed that the microsomal content of P450IIIA2 was reduced in hepatic fractions of IFN-treated rats. Furthermore, the hepatic content of hybridizable mRNA cor-

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ABBREVIATIONS: IFN, interferon; P450, cytochrome 450; androstenedione, androst-4-ene-3,17-dione; PCN, pregnenolone 16α-carbonitrile; n-rat IFNα/β, naturally derived IFNα/β; r-rat IFNγ, recombinant rat IFNγ; IgG, immunoglobulin G; SDS, sodium dodecyl sulfate.

responding to P450IIIA2 was found to be decreased to a similar extent. Additional experiments were performed in an attempt to clarify whether IFN altered P450IIIA expression by mechanisms involving known steroid regulators of this gene subfamily.

The P450IIIA subfamily (11) is responsible for microsomal steroid 6β-hydroxylation (12, 15). Representatives of this family have been found in all mammalian species examined to date, including humans (16, 17). An inducible member of this gene subfamily, termed P450IIIA1 (clone PCN1), is not present in untreated rat liver and may correspond to one or more immunologically-related P450 preparations (15). These have been isolated from rats induced with phenobarbital, PCN, or troleandomycin; they have been variously named P450PCN-E, PB-2a, P450p, PCNa, and PCNb (12, 16, 18). Since there is evidence of more than one inducible protein in the P450IIIA subfamily (19), the recommended notation P450IIIA1 (11) should not be taken to represent a single enzyme. Antibodies to these preparations cross-react with a protein termed P450IIIA2, which is a major constitutive P450 in male rat liver. A P450IIIA2 cDNA (clone PCN2; 89% homologous to PCN1) has been isolated from untreated male rat liver (15); a recently purified protein, P450PB-1, may correspond to this cDNA (20). P450IIIA2, which can be induced by phenobarbital but not by PCN, is expressed in immature rats of both sexes and is suppressed at puberty in females but not in males (12, 15). Androgens and growth hormone are both known to regulate expression of P450IIIA2 (12, 20).

Materials and Methods

Materials. Pregnenolone 16α-carbonitrile was a gift from Searle and Co., Chicago, IL. [4-^{14}C]Androstenedione (59 mCi/mmol) was purchased from Amersham Australia. Testosterone, unlabeled androstenedione, and 6β-hydroxy- and 16α-hydroxyandrostenedione, as well as all other biochemicals, were obtained from Sigma Chemical Co., St. Louis, MO. 7α-Hydroxyandrostenedione was obtained from Professor D. Kirk and the MRC Steroid Reference Collection, Queen Mary's College (London, United Kingdom). 16β-Hydroxyandrostenedione was prepared enzymatically as previously described (8). [γ^{32}P]ATP (3000 Ci/mmol) and GeneScreen Plus nylon filters were from New England Nuclear, Boston, MA. Recombinant rat interferon γ (r-rat IFNγ) was derived from Chinese hamster ovary cells and purified by monoclonal antibodies as described elsewhere (21). Naturally derived rat IFNα/β (n-rat IFNα/β) induced by Sendai virus was purified from a transformed rat embryonic cell line as previously reported (22). An oligonucleotide probe unique for P450IIIA2 cDNA, as described by Gonzalez *et al.* (15), was synthesized by Bioquest Ltd (Sydney, Australia); dephosphorylated oligodeoxythymidylic acid was from Pharmacia Australia.

Animal treatments. Adult male and female Wistar rats (200–250 g) were raised at the Westmead Hospital animal facility. They were housed in plastic cages and allowed free access to food and water.

Initial dosing and temporal studies with r-rat IFNγ were conducted in male rats to determine the optimal dose schedule for decreasing P450 levels and catalytic activities. Subsequent experiments employed 7.5×10^5 units of r-rat IFNγ given as a single intramuscular injection 24 hr prior to sacrifice; controls received vehicle (H_2O). In the experiment examining the effect of n-rat IFNα/β, male rats were given 5×10^5 units of IFN or vehicle 24 hr before sacrifice. In the study examining the effect of IFN on steroid induction of P450IIIA gene products, adult female rats were administered either r-rat IFNγ (7.5×10^5 units) or vehicle intramuscularly 24 hr before sacrifice. One hour after receiving IFN, each animal received PCN (400 mg/kg in 0.15 M NaCl and 0.005% Tween 80) intraperitoneally.

Microsomal steroid hydroxylase and serum sex steroid assays. Blood was taken from the vena cava, and the livers were removed from rats anesthetized with ether. Sections of liver were either stored in liquid nitrogen for subsequent isolation of RNA or were used for the preparation of microsomes by differential ultracentrifugation and then kept at −70° until used (8). Microsomal protein concentration (23) and P450 level (24) were determined by standard methods. Androstenedione hydroxylase activity was assayed in male and female microsomes as previously described, with minor modifications (8, 25). Separation of hydroxyandrostenedione metabolites was by thin layer chromatography (8). Serum estradiol and testosterone were determined with direct radioimmunoassay kits (CIS, Gif Sur Mur, France).

Immunoreactivity. Purification of P450IIIA from phenobarbital-treated rats was as previously described (26). Rabbits were immunized with P450IIIA protein, and the IgG fractions were isolated (27). The P450IIIA antibody recognizes P450IIIA2 and can therefore be used to immunoquantitate P450IIIA2 in microsomes from untreated male rat liver (27).

Hepatic microsomes (33 μg of protein per lane) were subjected to electrophoresis on SDS-polyacrylamide gels by established methods (28). Lanes containing standard amounts of pure P450IIIA (0.03–1.7 μg of protein) were also electrophoresed. Proteins were transferred to nitrocellulose sheets and then immunoblotted with anti-P450IIIA IgG (1:500 dilution) (28). Sheets were then incubated with a combination of ^{125}I-labeled and peroxidase-labeled donkey anti-rabbit IgG, visualized, and subjected to gamma counting.

RNA isolation and blot analysis. Total RNA was isolated from rat livers by the vanadyl ribonucleoside method (29). RNA (10 μg) was either separated electrophoretically on 2.0 M formaldehyde–1% agarose gels with subsequent blotting or bound to nylon filters (GeneScreen Plus) by using a Bio-Rad Bio-Dot SF apparatus (30).

Oligonucleotide probes for hybridization were labeled with [γ-^{32}P] ATP. Prehybridization was in Church and Gilbert buffer [1 M sodium phosphate buffer (pH 7.0), 7% SDS, 10% bovine serum albumin, 0.1 mM EDTA] at 50° for 2 hr; hybridization was carried out overnight under the same conditions. The filters were washed at 55° in 3× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate). Filters were then autoradiographed overnight at −70° (using Du Pont Cronex Lightning-Plus intensifying screens). To confirm equal loading of RNA, slot blot filters were stripped and rehybridized with [γ-^{32}P]ATP-labeled dephosphorylated oligodeoxythymidylic acid under the same conditions, except that washing was at 35° in 2× SSC containing 0.1% SDS. Quantitation of the oligonucleotides was by scanning with an LKB Ultroscan laser densitometer. The values of the integrated peaks were expressed as mean absorbance/μg of RNA.

Analysis of data. All results were expressed as mean ± standard deviation. Comparisons were performed by using Student's *t* test (unpaired, two-tailed) with a *p* value of <0.05 considered to be statistically significant.

Results

From initial studies, it was determined that the optimal dose of r-rat IFNγ for producing a maximal reduction of P450 levels and activities was 7.5×10^5 units administered 24 hr before sacrifice (Table 1 and data not shown). However, repeated daily injections of r-rat IFNγ for 3 days did not appear to decrease P450 levels further relative to values determined at 24 hr (data not shown). There was no difference in serum alanine transaminase levels, hepatic light microscopic morphology, or total microsomal protein among any of the treatment or control groups (data not shown).

The androstenedione 6β-hydroxylase activity of hepatic microsomes from male rats treated with r-rat IFNγ was reduced to 60% of control values [$p < 0.005$ (Table 1)]. Similarly, r-rat IFNγ reduced the rate of formation of the relatively minor 16β-

TABLE 1

Cytochrome P450 and androstenedione hydroxylation in hepatic microsomes from rats pretreated with r-rat IFNγ or vehicle

Values are mean ± standard deviation of results from six individual microsomal fractions. Statistical significance (p) is indicated in parentheses. NS, Not significant.

Treatment[a]	Cytochrome P450	Androstenedione hydroxylation pathway:			
		16α	16β	6β	7α
	nmol/mg of protein	*nmol/min/mg of protein*			
Vehicle	1.05 ± 0.06	1.95 ± 0.36	0.23 ± 0.02	2.05 ± 0.38	0.38 ± 0.05
r-rat IFNγ	0.82 ± 0.06 (<0.0001)	1.68 ± 0.45 (NS)	0.19 ± 0.04 (<0.05)	1.22 ± 0.35 (<0.005)	0.35 ± 0.11 (NS)

[a] Rats were injected with either r-rat IFNγ (7.5×10^6 units) or vehicle (H_2O) 24 hr before sacrifice.

TABLE 2

Cytochrome P450 and androstenedione hydroxylation in hepatic microsomes from rats pretreated with n-rat IFNα/β or vehicle

Values are mean ± standard deviation of results from six individual microsomal fractions. Statistical significance (p) is indicated in parentheses. NS, Not significant.

Treatment[a]	Cytochrome P450	Androstenedione hydroxylation pathway:			
		16α	16β	6β	7α
	nmol/mg of protein	*nmol/min/mg of protein*			
Vehicle	0.91 ± 0.07	2.33 ± 0.44	0.40 ± 0.08	2.43 ± 0.60	0.26 ± 0.03
n-rat IFNα/β	0.72 ± 0.07 (<0.001)	2.04 ± 0.19 (NS)	0.30 ± 0.05 (<0.05)	1.71 ± 0.10 (<0.05)	0.26 ± 0.03 (NS)

[a] Rats were injected with either n-rat IFNα/β (5×10^5 units) or vehicle (H_2O) 24 hr before sacrifice.

TABLE 3

Cytochrome P450 and androstenedione hydroxylation in hepatic microsomes from PCN-treated female rats given either r-rat IFNγ or vehicle

Values are mean ± standard deviation of results from six individual microsomal fractions; apparent differences are not significant.

Treatment[a]	Cytochrome P450	Androstenedione hydroxylation pathway:			
		16α	16β	6β	7α
	nmol/mg of protein	*nmol of product formed/min/mg of protein*			
Vehicle	1.35 ± 0.16	0.39 ± 0.09	0.70 ± 0.09	3.05 ± 0.53	0.53 ± 0.10
r-rat IFNγ	1.25 ± 0.12	0.38 ± 0.05	0.69 ± 0.04	3.21 ± 0.42	0.56 ± 0.05

[a] Adult female rats were injected intramuscularly with either r-rat IFNγ (7.5×10^6 units) or vehicle (H_2O) 24 hr before sacrifice. One hour after receiving IFN, each animal received PCN (400 mg/kg, intraperitoneally). In untreated female rat liver microsomes, the androstenedione 6β-hydroxylase and androstenedione 16β-hydroxylase activities were 0.23 ± 0.04 and 0.22 ± 0.04 nmol of product formed/min/mg of protein ($n = 6$), respectively.

hydroxyandrostenedione metabolite to 83% of the control values ($p < 0.05$). In contrast, the rates of androstenedione 16α- and 7α-hydroxylation were not different from those for control (Table 1).

The data in Table 2 demonstrate that n-rat IFNα/β had the same effect on microsomal steroid metabolism as r-rat IFNγ. Thus, the formation of 6β-hydroxyandrostenedione was reduced to 70% of control ($p < 0.05$), and the 16β-hydroxylation pathway activity was reduced to 75% of control activity ($p < 0.05$).

To determine whether IFN decreased the expression of P450IIIA1, female rats were coadministered PCN and r-rat IFNγ. The results in Table 3 confirm that PCN stimulated 6β-hydroxylase activity. For female rats, the only explanation for this effect is induction of P450IIIA1 (15). In contrast to the results with untreated male rats, IFN did not alter the rate of formation of any of the hydroxyandrostenedione metabolites in the PCN-treated female rats.

In untreated male rat liver, steroid 6β-hydroxylation is catalyzed almost exclusively by a male-specific P450 isozyme, P450IIIA2 (15, 20). In order to determine whether the reduction of androstenedione 6β-hydroxylase activity produced by IFN in male rats may be mediated by altered expression of P450IIIA2, microsomal levels of this protein were immunoquantitated in Western blots (Fig. 1). In r-rat IFNγ-treated hepatic microsomal fractions, levels of P450IIIA2 were decreased to 42% of control levels (6.2 ± 4.8 versus 14.6 ± 6.9 μg/mg of protein; $n = 6$ for each group, $p < 0.05$).

Northern blot analysis of adult male and female rat livers confirmed that P450IIIA2 mRNA is expressed in adult male, but not female, rats (data not shown). Slot blot hybridization was used to compare P450IIIA2 mRNA from IFN-treated and control male rat livers (Fig. 2). Thus, in r-rat IFNγ-treated liver, levels of P450IIIA2 mRNA were decreased to 35% of control levels (0.07 ± 0.03 versus 0.20 ± 0.05 absorbance units/μg of RNA; $n = 6$ for each group, $p < 0.0005$). Equal loading of RNA on the slot blot filters was confirmed by rehybridization with labeled dephosphorylated oligodeoxythymidylic acid (data not shown).

In view of the established role of sex hormones in the

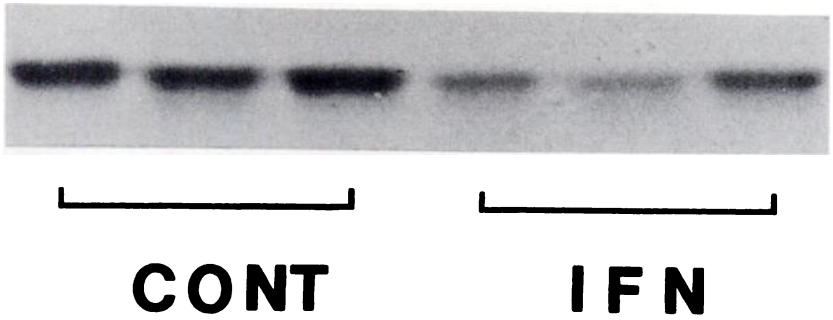

Fig. 1. Western immunoblot incubated with antiserum to P450IIIA proteins. The SDS-polyacrylamide gels were electrophoresed, the proteins were transferred to nitrocellulose sheets, and the blots were visualized as described in Materials and Methods. A representative immunoblot is shown. Male rats were treated with vehicle (CONT) or r-rat IFNγ (IFN) 24 hr before sacrifice.

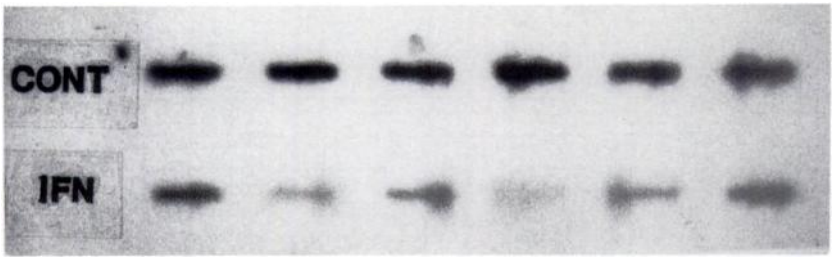

Fig. 2. Analysis of P450IIIA2 mRNA in control (CONT) and r-rat IFNγ-treated (IFN) male rat liver by using a specific P450IIIA2 oligonucleotide. Total liver RNA (10 μg) was bound to a nylon filter with a Bio-Dot SF device and hybridized with the P450IIIA2 oligonucleotide as described in Materials and Methods. The filter was exposed overnight at −70° with Du Pont Cronex Lightning-Plus intensifying screens.

regulation of certain P450s, serum testosterone and estradiol were measured in male rats given r-rat IFNγ. Twenty-four hours after IFN injection, serum testosterone levels were unchanged compared with control levels (0.48 ± 0.78 versus 0.52 ± 0.48 ng/ml; $n = 12$ for each group, NS). Similarly, there was no difference in serum estradiol levels in rats given r-rat IFNγ compared with control levels (23 ± 12 versus 16 ± 10 pg/ml; NS).

Discussion

The results from this study provide strong evidence that IFN differentially decreases the activities and levels of specific P450 isozymes in male rats. We previously demonstrated in male rats that administration of a lower dose of r-rat IFNγ (2×10^5 units) 24 hr before sacrifice reduces hepatic microsomal P450 levels and decreases formation of the relatively minor 16β-hydroxyandrostenedione metabolite (8). This suggested that the microsomal content of the androstenedione 16β-hydroxylase may be reduced by r-rat IFNγ. The steroid 16β-hydroxylase is most likely catalyzed by two isozymes, P450IIB1 and P450IIB2 (13); these phenobarbital-inducible proteins are present only at low levels in untreated male rat liver. In this study, these findings have been confirmed and extended by using a larger dose (7.5×10^5 units) of r-rat IFNγ, which reduced the formation of both the 16β- and 6β- hydroxyandrostenedione metabolites. However, unlike the 16β-hydroxylase, the androstenedione 6β-hydroxylase P450IIIA2 is a quantitatively important P450 in untreated male rat liver. The major finding of this study is that the IFN-mediated reduction of 6β-hydroxylase activity is due to decreased levels of the P450IIIA2 apoprotein. Moreover, hybridizable mRNA corresponding to P450IIIA2 was reduced to a similar extent as P450IIIA2 apoprotein (to about 40% of control level), suggesting that decreased apoprotein levels are a consequence of reduced transcription and/or altered posttranscriptional processes.

The decrease in androstenedione 6β-hydroxylase activity is less than expected on the basis of the decrease in P450IIIA2 apoprotein and mRNA. However, in untreated male rat liver, up to 15% of microsomal steroid 6β-hydroxylase activity is catalyzed by proteins other than P450IIIA2 (12). Other P450 isoforms with low androstenedione 6β-hydroxylase activities include P450IIC13, P450IIA2, P450IA1, and P450IA2 (14, 31, 32). Failure of IFN to down regulate these other isoforms may explain the higher than expected androstenedione 6β-hydroxylase activity.

Earlier studies have produced conflicting data concerning the mechanism whereby IFN reduces levels of hepatic P450. Ghezzi and co-workers suggested that after the induction of xanthine oxidase by IFN, superoxides are generated which subsequently destroy P450 (33). However, Mannering *et al.* suggested that the induction of xanthine oxidase and the loss of P450 following IFN administration were coincidental rather than causally related phenomena (34). Other studies with IFN inducers have suggested that P450 levels may be lowered as a result of altered heme turnover (9). More recently, Gooderham and Mannering demonstrated indirectly that IFN inducers may decrease P450 apoprotein synthesis as well as increase its degradation (10). This study provides direct evidence that IFN selectively decreases the activities of individual P450 isozymes. Reduced P450IIIA2 activity and apoprotein can be attributed to decreased levels of P450IIIA2 mRNA.

IFN is known to reduce expression of certain oncogenes by mechanisms involving down regulation of specific mRNAs (35, 36). Decreased expression of certain oncogenes in mouse cells by murine IFNγ appeared to be regulated at the transcriptional level (35). However, reduced levels of c-*myc* mRNA in Daudi cells following IFN administration was not associated with an alteration in the rate of c-*myc* transcription but, rather, reflected decreased half-life of c-*myc* mRNA (36). It remains to be established whether decreased P450IIIA2 mRNA following IFN administration is a consequence of altered P450IIIA2 transcription or a consequence of posttranscriptional events.

Of the three IFN subclasses, IFNα and IFNβ are most related, sharing considerable sequence homology, acid stability, heat stability, inducibility by viruses or double-stranded RNA, and a common receptor (37). IFNγ does not share these features, and so it seemed important to determine whether all rat IFNs had similar effects on hepatic microsomal P450. In this study, it was demonstrated that the rate of formation of the 6β- and 16β-hydroxylated metabolites of androstenedione in hepatic microsomes was affected to the same degree by n-rat IFNα/β as by r-rat IFNγ. This suggests that although these different IFNs act at distinct cellular receptors, they modulate similar changes to hepatic microsomal drug metabolism.

The expression of P450IIIA2 in untreated male rat liver is subject to hypothalamic-pituitary-gonadal control; both growth hormone and androgens are important for continued expression after puberty (12, 38). Reduced serum levels of sex steroids have been reported in patients receiving IFN (39). However, in the current study, serum sex steroids in rats were unchanged following IFN treatment. This finding was expected since the catalytic activity of P450IIC11 (the male-specific androstenedione 16α-hydroxylase), which is also regulated by androgens, was not affected by IFN.

The expression of P450IIIA1, which is closely related to P450IIIA2 and which is normally absent in untreated liver in both male and female rats, can be induced by the synthetic steroid PCN (12, 15). To assess whether IFN also decreased the expression of P450IIIA1, adult female rats [which normally do not express P450IIIA2 (15, 20)] were administered IFN followed by PCN. As expected, microsomal steroid 6β-hydroxylase activity was induced by PCN treatment. However, IFN

failed to reduce P450IIIA-dependent androstenedione 6β-hydroxylase activity in PCN-treated animals. This supports an earlier observation that in female rats, the induction of P450 by PCN could not be inhibited by the IFN inducer, polyriboinosinic·polyribocytidylic acid. However, in the same study, another IFN inducer, tilorone, impaired the induction of P450 by PCN (40). One interpretation of the findings from this study is that IFN may not decrease the expression of the steroid-inducible P450IIIA1. However, as it is possible that P450IIIA1 reflects several immunologically related steroid-inducible enzymes (17), IFN could have differing effects on individual contributing proteins.

In PCN-treated female rats, an observed increase in testosterone 16β-hydroxylase activity reflects induction of P450IIIA1 (41). In this study, the increased rate of formation of the 16β-hydroxyandrostenedione metabolite by PCN is probably a result of the same mechanism. However, IFN had no effect on androstenedione 16β-hydroxylase activity in PCN-induced female rats, in contrast to the decrease in activity it produced in male rats. The failure of IFN to suppress steroid 16β-hydroxylation activity in PCN-treated female rats could be explained by the major contribution of P450IIIA1 in this situation; unimpaired P450IIIA1 activity could have masked a minor decrease in the other steroid 16β-hydroxylase, P450IIB1 and P450IIB2.

These findings may be clinically relevant because members of the P450IIIA gene subfamily have been found in the liver of a number of mammalian species (16). Human P450IIIA orthologs [variously named P450HLp, $P450_{NF}$ and P450PCN3 (11, 17)] are quantitatively important hepatic P450s and are responsible for the 6β-hydroxylation of steroids, as well as the oxidation of such therapeutic agents as quinidine, nifedipine, cyclosporin A, and erythromycin (42). It is a reasonable prediction from the results of this study that the metabolism of therapeutic agents by the P450IIIA gene subfamily may be impaired by IFN. Direct studies in both rodents and humans should now be performed to clarify this issue.

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