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Testosterone-mediated regulation of mouse renal cytochrome *P*-450 isoenzymes

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We have studied the extent to which mouse renal cytochrome *P*-450 isoenzymes are sexually differentiated, and the factor(s) regulating this dimorphism. Intriguingly, sex differences were not seen in the expression of a single cytochrome *P*-450 enzyme, but were observed in the expression of all *P*-450 isoenzymes detectable, encoded by six gene families or sub-families. This effect was mediated by testosterone, which had the capacity to both induce and repress *P*-450 gene expression, and which was independent of growth hormone. The changes in protein content were mirrored in all but one case by changes in the levels of mRNA, indicating that these genes contain hormone-responsive elements. These findings are consistent with numerous reports of sex differences in the susceptibility of the mouse kidney to the toxic and carcinogenic effects of drugs and environmental chemicals, many of which are metabolized to cytotoxic products by the cytochrome *P*-450-dependent mono-oxygenases. These data imply that circulating androgen levels will be an important factor in susceptibility of the kidney to toxic or carcinogenic compounds which require metabolic activation.

INTRODUCTION

The cytochrome *P*-450 mono-oxygenases are distributed widely in Nature and catalyse the insertion of an atom of molecular oxygen into the substrate. In mammals, these proteins constitute a supergene family, which, on the basis of sequence similarity and chromosomal location, has been divided into families and sub-families (Wolf, 1986; Nebert *et al.*, 1989). These enzymes are predominantly located in the liver, but are also present in all other tissues (Aström & De Pierre, 1986; Guengerich, 1987). Cytochrome *P*-450 enzymes will collectively metabolize a wide variety of drugs and foreign compounds, and as such play a vital role in host defence against chemical insult. Unfortunately, some reactions catalysed by these enzymes can result in activation of the compounds concerned, leading to chemical toxicity and cancer (Oesch, 1984).

It is therefore important that the factor(s) which regulate cytochrome *P*-450 gene expression are elucidated. Previous work on the rat liver *P*-450 enzymes has demonstrated the role of growth hormone (GH) in determining hepatic *P*-450 levels (Mode *et al.*, 1982, 1983; Blanck *et al.*, 1986; Skett, 1987; Zaphiropoulos *et al.*, 1989). GH secretion is sexually differentiated in the rat (Eden, 1979): in male rats, GH secretion is episodic, whereas in females, GH output is more continuous. This dimorphism is believed to be responsible for the distinct *P*-450 isoenzyme profiles seen in males and females; generally, hepatic xenobiotic metabolism is greater in male than in female rats. In mice, a similar situation exists, although the orientation of the effect is opposite, i.e. female hepatic xenobiotic metabolism is greater than that found in males (MacLeod & Shapiro, 1989). Although direct measurement of GH profiles has yet to be carried out in mice, it has been possible to alter experimentally the GH secretion pattern, either by using transgenic mice or through naturally occurring GH-deficient strains (Norstedt & Palmiter, 1984). These studies have demonstrated that modulation of GH secretion alters the expression of proteins and enzyme activities known to be sexually differentiated in the mouse liver.

For a number of reasons, the kidneys represent a particular target for the toxic effects of foreign compounds (Hook *et al.*, 1979; Lock, 1987). This tissue, on average, accounts for only 1% of body weight, but receives 25% of the resting cardiac output. This, together with the inherently concentrative nature of the organ, results in extremely high concentrations of drugs and environmental chemicals in the proximal tubules (Hook *et al.*, 1979).

The profound sexual differentiation of the mouse kidney in terms of toxicological response is believed to be due to the sexual dimorphism of renal cytochrome *P*-450 enzymes. In order to investigate the degree of sexual differentiation of mouse renal cytochrome *P*-450 isoenzymes, and the potential role of GH in their regulation, the mouse strain 'little' was used (Eicher & Beamer, 1976). This strain, derived from C57BL/6, is deficient in the pituitary receptor for GH-releasing factor (Clark & Robinson, 1985; Jansson *et al.*, 1986), and as a consequence the homozygote (*lit/lit*) has only 5–10% of normal circulating GH levels (Cheng *et al.*, 1983). The heterozygote (*lit/+*) has been shown to possess the wild-type phenotype (Beamer & Eicher, 1976). No other biochemical or endocrinological abnormality has been described in this strain. For these reasons, the 'little'

Abbreviations used: GST, glutathione *S*-transferase; GH, growth hormone.

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mouse provides an ideal model for studying the role of GH in the regulation of *P*-450 gene expression, thus obviating the need for hypophysectomy as a means of removing the influence of GH.

In order to establish the extent of sexual differentiation of cytochrome *P*-450 enzymes in the mouse kidney, and the regulatory factor(s) involved, we have determined the levels of six distinct gene families or sub-families.

MATERIALS AND METHODS

Chemicals

Unless otherwise indicated, all chemicals were purchased from either Sigma Chemical Co. Ltd., Poole, Dorset, U.K., or BDH Ltd., Glasgow, U.K., and were of analytical grade or better. Nitrocellulose was obtained from Schleicher and Schull, Dassell, Germany. Resorufin analogues were purchased from Molecular Probes Inc., Junction City, OR, U.S.A. Recombinant human GH (Genotropin) was a gift from Kabi-Vitrum (U.K.). Human GH has been shown previously to be physiologically active in C57BL/6 mice at the dosage used in these experiments (Norstedt & Palmiter, 1984).

Animals

Adult male and female C57BL/6 (+/+) mice, the GH-deficient strain 'little' (*lit/lit*) and the heterozygous strain (*lit/+*) were obtained from Charles River, Wilmington, MA, U.S.A., and were fed *ad libitum* on standard animal diet. The mice (six of each sex per treatment group) were given intraperitoneal injections of the cytochrome-*P*-450-inducing agents phenobarbital (80 mg/kg per day for 3 days in phosphate-buffered saline) or dexamethasone (100 mg/kg per day for 4 days in corn oil). Control animals received injection vehicle only.

A further set of 'little' mice (three of each sex per treatment group) received either testosterone propionate, subcutaneously in corn oil at 4 mg/kg body weight, or recombinant human GH (Genotropin), subcutaneously in sterile water in twice-daily doses of 5μg; the injections were separated by approx. 12 h to simulate the male pattern of GH secretion. Treatment lasted 7 days, and control animals received injection vehicle only.

Male C57BL/6 mice were sham-operated or castrated at 8 weeks of age. At 11 weeks, the animals were treated daily with testosterone propionate, administered subcutaneously at 2 mg/kg or 4 mg/kg body weight, for 11 days. Intact female C57BL/6 mice also received testosterone propionate, as outlined above for males. Control animals received vehicle (corn oil) only for the same period. Each treatment group consisted of six mice.

Mice of the strain *hpg* were generously supplied by the M.R.C. Brain Metabolism Unit, Edinburgh, U.K. This mouse strain has a gonadotropin-releasing-hormone deficiency, with a consequent reduction in pituitary content and circulating levels of luteinizing hormone and follicle-stimulating hormone (Cattanach *et al.*, 1977). As a result, the ovaries and testes fail to develop postnatally.

After the last day of treatment, the animals were starved overnight before being killed by cervical dislocation. The organs were removed rapidly, rinsed in phosphate-buffered saline and frozen on solid CO_2. Tissue was stored at −70 °C until required.

Preparation of microsomes

Kidney microsomal samples were prepared by differential centrifugation from pooled tissue as described previously (Meehan *et al.*, 1988). Microsomes were finally resuspended in 10 mM-potassium phosphate buffer (pH 7.4)/1% (w/v) KCl/1 mM-EDTA/0.25M-sucrose, and stored at a concentration of 10–20 mg/ml at −40 °C.

Protein content was measured according to the method of Lowry *et al.* (1951).

Cytochrome *P*-450 content was estimated by measuring the reduced carbon monoxide difference spectrum as described by Omura & Sato (1964).

Metabolism of *P*-450 substrates

The metabolism of the cytochrome *P*-450 substrates benzyloxyresorufin and coumarin were determined by measuring the rate of generation of the fluorescent products resorufin and 7-hydroxycoumarin respectively (Burke *et al.*, 1985). The metabolism of lauric acid was measured using ^{14}C-labelled substrate and by subsequent separation of the products by t.l.c. (Orton & Parker, 1982). This assay measures both 11- and 12-hydroxy lauric acid products.

Immunoblotting

SDS/PAGE was carried out according to the method of Laemmli (1970), using 9% separating gels.

Immunoblotting was performed essentially as described by Towbin *et al.* (1979), with modifications according to Lewis *et al.* (1988). After separation, proteins were transferred electrophoretically to nitrocellulose and probed with various antisera to rat liver cytochrome *P*-450s. Antibodies to the purified enzymes were isolated as described previously (Wolf *et al.*, 1984, 1986). These antibodies have been used previously in immunoblotting studies with mouse microsomal samples (Meehan *et al.*, 1984). In addition, the isoenzyme specificities of the antisera have been demonstrated by immunoblot analysis with expressed human recombinant *P*-450 proteins (results not shown). Cytochrome *P*-450IVA1 was isolated according to the method of Tamburini *et al.* (1984), and was of high purity, running as a single band on SDS/PAGE. Antibodies used were to *P*-450IA1, *P*-450IIA1, *P*-450IIB1, *P*-450IIC6, *P*-450IID1, *P*-450IIE1, *P*-450IIIA1 and *P*-450IVA1. (For nomenclature system, see Nebert *et al.*, 1989.)

After revealing the immunoreactive polypeptides using horseradish peroxidase-labelled second antibody, the signal was enhanced with ^{125}I-labelled Protein A (Amersham International) and subsequent autoradiography (Kodak X-Omat AR5 X-ray film) at −70 °C. Different exposure times were used in order to optimize the autoradiographic signal.

Northern blotting

RNA was isolated from kidney samples of male and female mice as described previously by Cox (1968). RNA concentration and purity were estimated spectrophotometrically, and RNA was tested for integrity and equivalence of loading by ethidium bromide staining of a denaturing formaldehyde gel. mRNA was separated and *P*-450 mRNA content was determined on denaturing formaldehyde gels using hybridization conditions described previously (Meehan *et al.*, 1984). Blots were

washed at 65 °C with 0.3 M-NaCl/0.03 M-trisodium citrate (pH 7.4). cDNA probes were labelled by the random-priming method (Rigby *et al.*, 1977; Feinberg & Vogelstein, 1983, 1984).

Human cDNA probes used were *P*-450IA1, *P*-450IIA3, *P*-450IIB1, *P*-450IIC6, *P*-450IID1, *P*-450IIE1 and *P*-450IIIA1. The *P*-450IVA1 cDNA probe was isolated from the rat (Earnshaw *et al.*, 1988). All probes were shown to be specific for their respective gene families in the mouse by their pattern of hybridization and by mapping in the recombinant inbred lines.

RESULTS

In our initial studies, we investigated the metabolism of certain model cytochrome *P*-450 substrates to see whether they were metabolized at different rates in male and female kidney samples (Fig. 1). In agreement with previous findings on the metabolism of compounds such as chloroform (Eschenbrenner, 1945; Pohl *et al.*, 1984), a marked sex difference was observed. In female mice, total cytochrome *P*-450 levels were only 25% of the male values, and the rates of metabolism of the substrates coumarin, benzyloxyresorufin and lauric acid were 11.5, 1.5 and 26% respectively of those measured in males. These data clearly demonstrate the wide spectrum of *P*-450 activities which are sexually differentiated in the mouse. Interestingly, the three substrates used have been shown to be metabolized by *P*-450 forms from distinct gene families or sub-families, i.e. the *P*-450IIA, *P*-450IIB and *P*-450IV sub-families (Bains *et al.*, 1985; Meehan *et al.*, 1988; Miles *et al.*, 1989).

Western blot analysis, using antibodies to distinct *P*-450 families, was used to identify the proteins involved in such a sexual dimorphism (Fig. 2). In addition to untreated (control) mice, a group of animals was treated with the *P*-450-inducing agents phenobarbital and dexamethasone in order to induce the expression of *P*-450 forms which would otherwise be difficult to detect.

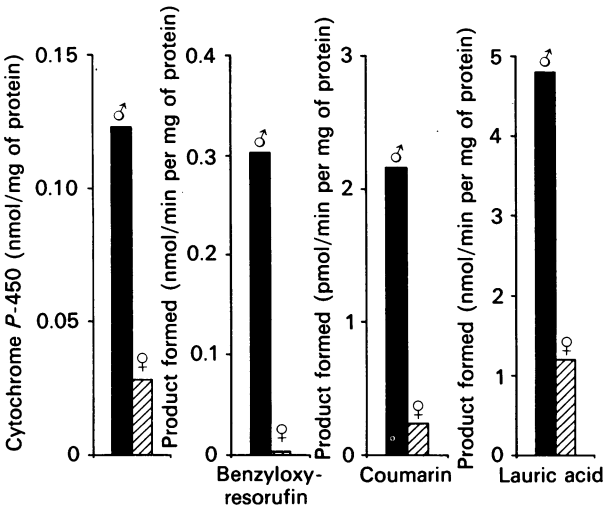

Fig. 1. Sexual differentiation of cytochrome *P*-450-dependent mono-oxygenases in mouse kidney

Microsomal *P*-450 content and substrate metabolism were measured in kidney microsomes from untreated wild-type (+/+) C57BL/6 male and female mice. Results are expressed as nmol or pmol of product formed/min per mg of microsomal protein, and represent the means of three separate determinations.

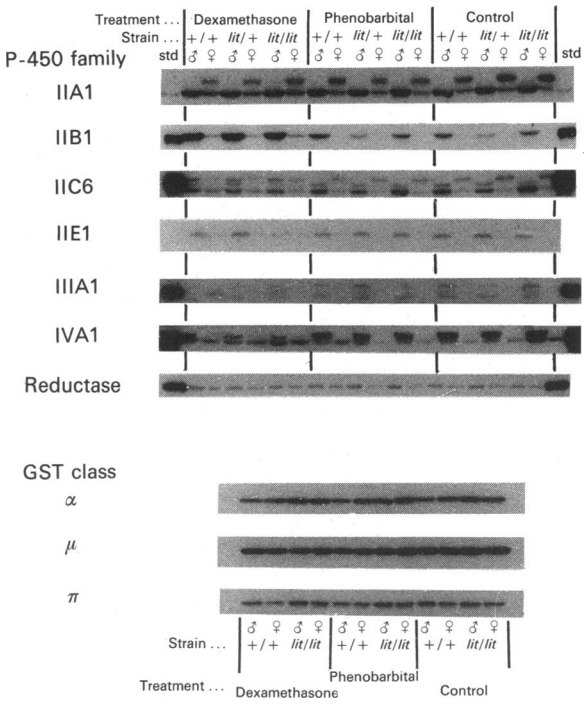

Fig. 2. Cytochrome *P*-450 reductase and GST isoenzyme expression in mouse kidney

Microsomal proteins (30 μg) and cytosolic proteins (15 μg) from control or phenobarbital- or dexamethasone-treated mice, and *P*-450 standards (0.4 pmol) were separated by SDS/PAGE, immunoblotted and probed with polyclonal antisera raised to rat liver cytochrome *P*-450 isoenzymes, *P*-450 reductase and GSTs. No bands were detected on Western blots other than those shown. The standards (std) used were *P*-450IIA1 (48 kDa), *P*-450IIB1 (52 kDa), *P*-450IIC6 (51 kDa), *P*-450IIIA1 (51 kDa), *P*-450IVA1 (51 kDa) and *P*-450 reductase, isolated from rat liver. No *P*-450IIE standard was available.

The Western blots demonstrated that *P*-450s from almost all the known gene families involved in foreign compound metabolism are expressed in the kidney, and up to eleven cytochrome *P*-450 isoenzymes from six gene families or sub-families were sexually differentiated in a striking manner. In certain cases, several members of a gene family exhibited differences. In the *P*-450IIA sub-family, an isoenzyme is expressed only in females, but the level of expression of the lower-molecular-mass form is greater in males. Interestingly, a different antibody preparation, raised to a mouse protein of the *P*-450IIA sub-family, gave the same banding pattern but in addition recognized a second male-specific band of intermediate molecular mass (results not shown). Polyclonal antibodies to rat *P*-450IIA1 will inhibit completely coumarin hydroxylase activity in both mouse and human liver microsomes (results not shown). Thus it may be assumed that this *P*-450 isoenzyme alone carries out the 7-hydroxylation of coumarin, and that the differences in *P*-450IIA protein levels (Fig. 2) will explain the observed dimorphism in coumarin hydroxylase activity (Fig. 1). Similarly, the association of lauric acid hydroxylase

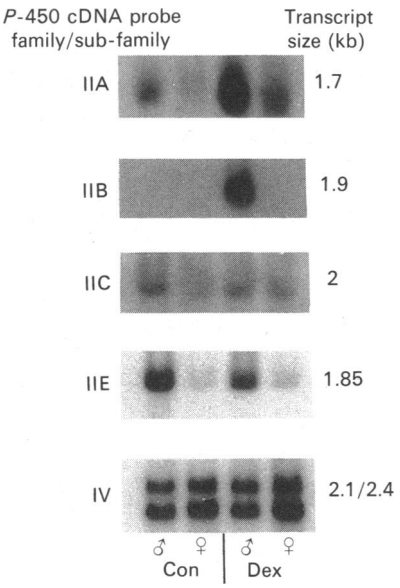

Fig. 3. Cytochrome *P*-450 mRNA analysis of male and female mouse kidney samples

RNA was isolated and Northern blotting carried out as described in the Materials and methods section. The following cDNA probes were used: *P*-450IIA3, *P*-450IIB1, *P*-450IIC6, *P*-450IIE1 and *P*-450IVA1. Con, control; Dex, dexamethasone-treated male and female (+/+) C57BL/6 mice.

activity with *P*-450IVA1 is well documented (Bains *et al.*, 1985). Two *P*-450IV-related proteins were expressed at much higher levels in males, thus explaining the higher rate of lauric acid metabolism in this sex. These proteins correspond to the two *P*-450IVA transcripts observed on Northern blots (Fig. 3). In four other mouse species, C3H, DBA, C57L and AKR, sexual differentiation of kidney microsomal lauric acid metabolism was also evident (results not shown). The metabolism of benzyloxyresorufin is associated with proteins of the *P*-450IIB gene family (Meehan *et al.*, 1988), and a protein within this family, which is expressed at higher levels in males and is strongly induced by dexamethasone but not phenobarbital, is essentially absent from female samples. Both male- and female-specific bands were recognized by antibodies to the *P*-450IIC gene family; also, dexamethasone appeared to induce in male mice, and suppress in females, the expression of a high-molecular-mass protein. A single protein associated with the *P*-450IIE family was detected in male, but not in female, samples. Although members of the *P*-450III family were expressed at very low levels, it appears that two, possibly three, members of this gene family are expressed in males, but not in females. Interestingly, proteins of this gene family did not appear to be induced by dexamethasone in the kidney, which is contrary to the regulation of these proteins in the liver. *P*-450 from the *P*-450IID family could not be detected in the mouse kidney (results not shown).

In order to establish whether other drug-metabolizing enzymes are also sexually differentiated in the mouse kidney, Western blots were carried out using antibodies to cytochrome *P*-450 reductase, and to the cytosolic glutathione *S*-transferases (GST) from three gene families, Alpha, Mu and Pi (Hayes *et al.*, 1983; Stockman *et al.*, 1987). No evidence for sexual differentiation of the GST families in the kidney was seen (Fig. 2). A similar result was obtained for the microsomal GST (results not shown). *P*-450 reductase levels were equivocal (Fig. 2), suggesting a slight sexual dimorphism, although this was much less dramatic than that demonstrated by the *P*-450 isoenzymes.

Northern blot analysis was carried out to determine whether the changes in protein levels were mirrored by changes in the mRNA content (Fig. 3). In almost all cases, a higher level of mRNA was measured in males than in females, in agreement with the higher protein levels seen on Western blots. These data confirm the sexual differentiation of *P*-450s from the gene families studied, and also indicate that regulation may be at the transcriptional level. One anomaly was the finding of no sex difference in mRNA levels using the *P*-450IVA1 cDNA probe. The reason for this is unclear, but may indicate some other form of regulation. Samples were also probed with *P*-450IA1, *P*-450IID and *P*-450IIIA1 cDNAs, but no hybridization to these probes could be detected (results not shown).

The profile of *P*-450 isoenzymes in the GH-deficient 'little' mice (*lit/lit*) was identical with that found in the heterozygous (*lit*/+) or normal (+/+) strains, indicating that GH is not involved in the sexual differentiation of *P*-450 expression in the kidney (Fig. 2). This was further substantiated by treating female *lit/lit* mice with GH in a male pattern; the renal *P*-450 isoenzyme profile remained that of a female (Fig. 4). These data are in

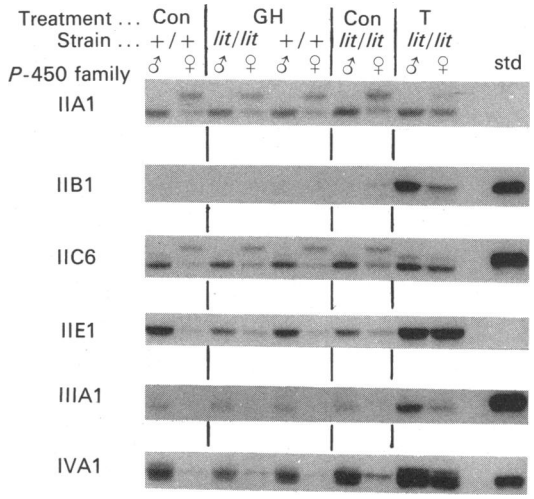

Fig. 4. Effects of GH and testosterone on cytochrome *P*-450 expression

Microsomal protein (30 μg) and *P*-450 standards (0.4 pmol) were separated by SDS/PAGE, immunoblotted and probed with polyclonal antisera raised to rat liver cytochrome *P*-450 isoenzymes. The standards (std) used were *P*-450IIA1, *P*-450IIB1, *P*-450IIC6, *P*-450IIIA1 and *P*-450IVA1, isolated from rat liver. No *P*-450IIE standard was available. The *P*-450 level in the control and GH-treated microsomal samples was so low as to prevent clear visualization with the *P*-450IIB1 and *P*-450IIA1 antisera. Con, control animals receiving corn oil vehicle only; GH, growth-hormone-treated mice; T, testosterone propionate-treated mice. Further details are given in the Materials and methods section.

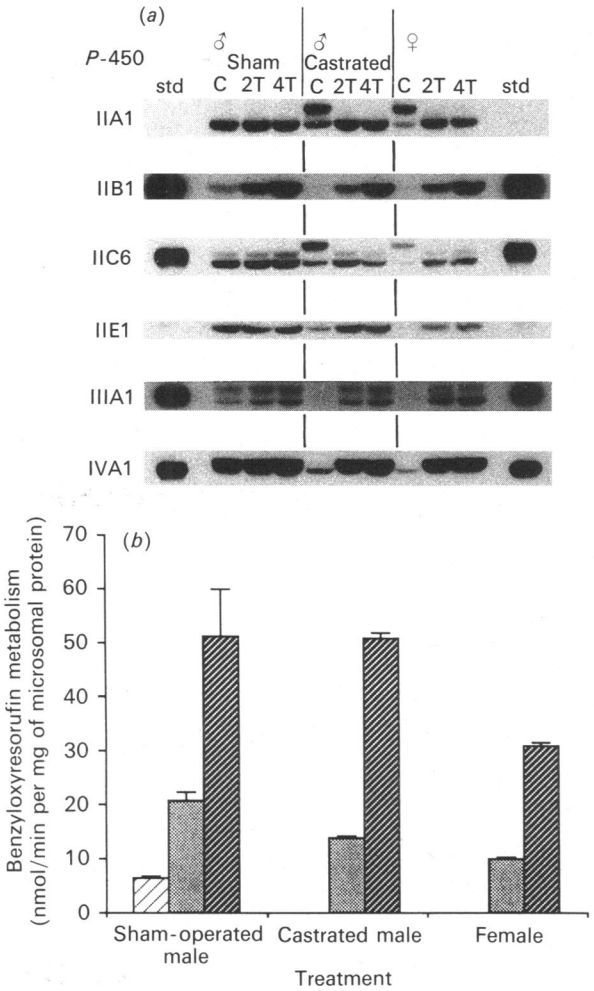

Fig. 5. Regulation of mouse kidney cytochrome *P*-450 expression by testosterone

(*a*) Microsomal protein (30 μg) and *P*-450 standards (0.4 pmol) were separated by SDS/PAGE, immunoblotted and probed with polyclonal antisera raised to rat liver cytochrome *P*-450 isoenzymes. The standards (std) used were *P*-450IIA1, *P*-450IIB1, *P*-450IIC6, *P*-450IIIA1 and *P*-450IVA1, isolated from rat liver. No *P*-450IIE standard was available. C, control animals receiving corn oil vehicle only; 2T and 4T, animals receiving a daily dose of testosterone propionate of 2 and 4 mg/kg body weight respectively, as described in the Materials and methods section. (*b*) Kidney microsomal benzyloxyresorufin-metabolizing activity in sham-operated and castrated male and intact female mice. ▨, Control; ▩, 2 mg of testosterone/kg; ▩, 4 mg of testosterone/kg. Details are given in the Materials and methods section.

contrast with the situation in the liver, where an altered expression of hepatic *P*-450 isoenzymes in the *P*-450IIB and *P*-450IIC gene families is observed in 'little' mice (results not shown).

As GH did not appear to mediate the differences observed, and in view of the known effects of testosterone on the toxicological response of the kidney, we investigated whether steroid hormones might be involved. Striking effects of castration and testosterone treatment were observed (Figs. 4 and 5). Castration of male mice converted their pattern of cytochrome *P*-450 expression to that seen in females, the male pattern being restored by treatment with testosterone (Fig. 5*a*). Treatment of female mice with testosterone resulted in suppression of the female *P*-450 profile and induction of the male pattern. These findings explain reports that testosterone sensitizes female mice to the nephrotoxic effects of compounds such as chloroform, and are consistent with previous reports that proteins from the *P*-450IIA and *P*-450IIE sub-families are regulated in the kidney by testosterone (Squires & Negishi, 1988; Hong *et al.*, 1989). Benzyloxyresorufin metabolism by kidney microsomes (Fig. 5*b*) from these mice reflected the results obtained from Western blotting with anti-*P*-450IIB antiserum. Castration of males suppressed the metabolism to female (undetectable) levels, whereas testosterone treatment of intact females or castrated males induced the metabolism from undetectable to male levels. Interestingly, the induction of *P*-450IIB by testosterone was extremely marked, in terms of both immunoreactivity on a Western blot (Fig. 5*a*) and of activity with benzyloxyresorufin (Fig. 5*b*), and appeared to be dose-dependent.

Treatment of female *lit/lit* mice with testosterone resulted in a male *P*-450 isoenzyme profile (Fig. 4). This suggests that testosterone is not operating via the pituitary and modulation of the pattern of GH levels. This is substantiated by the finding that the imposition of a male GH pattern on female *lit/lit* mice had no effect on the renal *P*-450 isoenzyme profile (Fig. 4). Western blotting of kidney microsomes from male and female *hpg* mice did not reveal sexual dimorphism in the renal *P*-450 isoenzyme profile, both males and females exhibiting the same ('female') pattern of *P*-450 expression (results not shown). Ovariectomy of female mice had no effect on the pattern of renal *P*-450 isoenzyme expression (results not shown); thus oestrogens are not involved in determining the female *P*-450 levels. The expression of cytochrome *P*-450 genes is therefore inducible or repressible by testosterone, acting either directly or indirectly.

DISCUSSION

There are numerous reports describing sex differences in susceptibility to environmental toxins and carcinogens, in animal models as well as in man. These differences are observed in a variety of tissues, but are perhaps best exemplified by studies in the liver and the kidney. In the latter case, it is remarkable that a host of compounds exhibit profound sex differences in their effects. For example, chloroform, which is activated to its nephrotoxic form, phosgene, by the cytochrome *P*-450 mono-oxygenase system, is highly toxic to the male mouse kidney, but is essentially non-toxic in females (Eschenbrenner, 1945; Pohl *et al.*, 1984). Other studies have demonstrated a greater incidence of renal neoplasms in male mice with a variety of compounds, including 2,4-dinitrotoluene, tris-(2,3-dibromopropyl) phosphate, 4,4′-dichlorophenyltrichloroethane, basic lead acetate and ethylnitrosourea (Van Esch & Kroes, 1969; Tomatis *et al.*, 1972; Vesselinovitch *et al.*, 1973; Reznik *et al.*, 1979; Hong *et al.*, 1980).

A wide variety of factors are important in determining the response of a tissue to a chemical toxin. The relative rate of metabolic activation of a compound by the cytochrome *P*-450-dependent mono-oxygenase system versus its detoxification have been considered to be of central importance (Oesch, 1984). Indeed, in the

kidney, sexual dimorphism in the rate of activation of nephrotoxins by cytochrome *P*-450s has been proposed as a mechanism for some of the toxicological differences observed (Hook *et al.*, 1979; Rush *et al.*, 1984; Lock, 1987). The implication from these studies is that cytochrome *P*-450s are regulated in the kidney in a sex-specific manner (Hawke & Welch, 1985). This possibility, however, is difficult to reconcile with the remarkably broad range of compounds exhibiting sexual differentiation, unless several cytochrome *P*-450 forms are sexually differentiated. The results presented in this paper demonstrate that *P*-450 genes from all gene families detectable in the kidney are subject to hormonal regulation, either directly or indirectly.

Immunoblots indicated that there may be eleven or more sexually differentiated *P*-450 forms expressed in the mouse kidney. The actual number of forms should, however, be interpreted with some caution, as antibodies to rat liver *P*-450 isoenzymes were used. There is therefore the possibility that the antibodies may cross-react with other gene families in the mouse. However, several lines of evidence indicate that this is not the case. The antibodies used have been shown to be specific inhibitors of mono-oxygenase reactions catalysed by specific *P*-450 forms in the mouse, and have been shown to distinguish between gene families based on the mode of regulation of the proteins which they recognize (Meehan *et al.*, 1988; R. R. Meehan & C. R. Wolf, unpublished work). In addition, the antibodies have also been shown to exhibit specificity towards recombinant human *P*-450 forms (L. Forrester, F. Gonzalez & C. R. Wolf, unpublished work).

A number of cellular enzymes have been reported to be present at higher levels in the male mouse kidney than in the female, and sexual dimorphism in subcellular organelles has also been reported (Koenig *et al.*, 1980). The molecular basis for these sex differences in many cases has not been studied in detail, although steroid hormones have been implicated (Koths *et al.*, 1972; Smith *et al.*, 1984). Studies of certain androgen-regulated genes (non-*P*-450) in the mouse kidney and other murine tissues have shown that the presence of the androgen receptor is required for testosterone to have its regulatory effect (Felder *et al.*, 1988), and that testosterone stimulates transcription (Watson & Paigen, 1988). There are preliminary reports describing areas of sequence similarity in the 5′ non-coding region of androgen-regulated genes from various rat and mouse tissues (Kandala *et al.*, 1985; Williams *et al.*, 1985), implying the presence of androgen-responsive elements. In the case of *P*-450s, it will be useful to establish whether testosterone is acting at the transcriptional level by stimulating *P*-450 mRNA synthesis. If this proves to be the case, then the presence of hormone-responsive elements in a large number of mouse *P*-450 genes from several gene families may be implied.

Hormonal regulation of *P*-450 genes in the kidney could well explain the increasing number of reports demonstrating sex differences in susceptibility to toxins and carcinogens. In humans, for example, Balkan Endemic Nephropathy, which has been associated with exposure to the fungal toxin ochratoxin A, believed to be a *P*-450 substrate, exhibits a sexual dimorphism in its incidence (Castegrano *et al.*, 1987). There is also a profound difference in the nephrotoxicity and carcinogenicity of this toxin in mice, which appears to be related to its rate of metabolic activation (Bendele *et al.*, 1985). Moreover, steroid hormones have been implicated in the aetiology of renal cancer, which occurs twice as frequently in men than in women (Bloom, 1971, 1973). This could relate to differences in cytochrome *P*-450 levels.

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