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SEX DIFFERENCES IN THE DIABETES-INDUCED MODULATION OF RAT HEPATIC CYTOCHROME P450 PROTEINS

C. R. BARNETT, S. RUDD, P. R. FLATT* and C. IOANNIDES†

Molecular Toxicology Group, Division of Toxicology, School of Biological Sciences, University of Surrey, Guildford, Surrey GU2 5XH; and *Biomedical Sciences Research Centre and Department of Biological and Biomedical Sciences, University of Ulster, Coleraine, Co. Londonderry BT52 1SA, Northern Ireland, U.K.

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Abstract—Sex differences in the diabetes-induced changes in hepatic cytochrome P450 proteins were investigated in rats treated with streptozotocin. Changes in specific cytochrome P450 proteins were monitored using diagnostic substrates and immunologically utilizing specific polyclonal antibodies. When expressed in terms of nmoles of total cytochrome P450, ethoxyresorufin *O*-deethylase activity was increased by treatment with streptozotocin, the extent of induction being the same in the two sexes. In contrast, lauric acid hydroxylase and ethylmorphine *N*-demethylase activities were induced only in the male rat. Finally, *p*-nitrophenol hydroxylase and pentoxyresorufin *O*-dealkylase were enhanced by the same treatment in both sexes, the effect being more pronounced in the male. These findings indicate that sex-specific changes in certain cytochrome P450 proteins exist in response to insulin-dependent diabetes but these cannot, however, be ascribed to sex differences in the severity of diabetes induced by streptozotocin since the degrees of hyperketonaemia and hyperglycaemia were the same in the two sexes. These are likely to reflect sex-specific differences in growth hormone and triglyceride levels in the diabetic animals.

The cytochrome P450-dependent mixed-function oxidase system is the principal catalyst of the oxidation of xenobiotics that gain entry into the living organism. Following such metabolism xenobiotics may be converted to readily excretable inert, polar metabolites or to reactive electrophiles which, if not effectively detoxicated, may interact with vital cellular macromolecules initiating a number of toxicological processes [1, 2]. One of the major characteristics of the mixed-function oxidase system is its unprecedented broad substrate specificity that it achieves by existing as a number of distinct families of which the first three (CYP1–CYP3) are involved in the metabolism of and induced by exposure to chemicals [3, 4]. Another family, namely CYP4, is also inducible by xenobiotics and, although no role in xenobiotic metabolism has been established, is closely associated with peroxisomal proliferators, a group of rodent epigenetic carcinogens [5].

These cytochrome P450 families are markedly influenced by an imbalance of circulating insulin. The levels and activities of proteins belonging to these four families are increased following the induction of insulin-deficient diabetes by streptozotocin [6–9]. Moreover, the CYP1 family is also enhanced by the hyperinsulinaemia that results following the transplantation of an insulin-secreting tumour, or following the injection of long-acting insulin to rats [10]. Such changes in cytochrome P450 proteins are of toxicological significance [11]. In the studies of chemically induced diabetes, sex differences have been described in some, but not all, drug metabolizing activities [12–14]. These findings indicate that sex-specific changes in certain cytochrome P450 proteins may exist in response to insulin-dependent diabetes.

The purpose of the present study is: (a) to establish whether sex differences occur in the diabetes-induced changes in specific cytochrome P450 proteins and (b) to consider possible mechanisms for such differences.

MATERIALS AND METHODS

Ethylmorphine (Rhône-Poulenc, Dagenham, U.K.) resorufin, and ethoxy- and pentoxyresorufins (Molecular Probes, Eugene, OR, U.S.A.), peroxidase-linked donkey anti-sheep IgG, peroxidase-linked donkey anti-rabbit IgG, *p*-nitrophenol, 3-methylcholanthrene, isoniazid, streptozotocin, lauric acid and all cofactors (Sigma Chemical Co., Poole, U.K.), phenobarbitone (BDH, Poole, U.K.), pregnenolone 16α-carbonitrile (Upjohn Co., Kalamazoo, MI, U.S.A.), and [^{14}C]lauric acid (Amersham International plc, Amersham U.K.) were all purchased. Sodium clofibrate was a gift from I.C.I. plc, Pharmaceuticals Division, (Macclesfield, U.K.). The purification of antigens and antibody production to cytochromes CYP1A1 and CYP2B1 have already been described [15]. Antibodies to CYP1 recognise both A1 and A2 proteins and similarly antibodies to P4502B1 recognise both B1 and B2 proteins as well as a third protein of lower relative molecular mass which is present in the liver of Wistar rats [16, 17]. Antibodies to CYP2E1, CYP3A1 and CYP4A1 were generous gifts from Dr D. Koop (Case Western Reserve University, School of Medicine, OH, U.S.A.), Dr C. R. Wolf (Imperial Cancer Research

† Corresponding author.

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Male and female Wistar albino rats (180–200 g) were obtained from the Experimental Biology Unit, University of Surrey. One group of each sex received a single intraperitoneal injection of streptozotocin (65 mg/kg), dissolved in 0.5 M citrate buffer pH 4.5, while a second group of each sex was treated with the buffer only and served as control. All animals were killed 21 days after commencement of the treatment. Livers were immediately excised and microsomal fractions prepared as described previously [18]. The following assays were carried out: ethoxyresorufin *O*-deethylase [19], pentoxyresorufin *O*-dealkylase [20], *p*-nitrophenol hydroxylase [21], ethylmorphine *N*-demethylase [22], total cytochrome P450 and b_5 [23] and protein using bovine serum albumin as standard [24]. The hydroxylation of lauric acid was determined by the TLC method of Parker and Orton [25] and represents the combined formation of 12(ω)- and 11(ω-1)-hydroxylauric acid metabolites. Plasma glucose [26], and 3-hydroxybutyrate and acetoacetate [27] concentrations were determined on terminal blood samples.

To serve as positive controls in the immunological studies for the induction of CYP1A, CYP2B, CYP3A and CYP4A proteins, groups of previously untreated male rats were treated with single daily intraperitoneal administrations of 3-methylcholanthrene (25 mg/kg), phenobarbitone (80 mg/kg), pregnenolone 16α-carbonitrile (100 mg/kg) and clofibrate (80 mg/kg), respectively, for 3 days, all animals being killed 24 hr after the last injection. Finally, to serve as a positive control for the CYP2E subfamily, a further group of animals were exposed to isoniazid (0.1% w/v in drinking water for 7 days).

For the immunoblot analysis, microsomal proteins were solubilized in 0.1 M phosphate buffer pH 7.4, containing emulgen (10% v/v), and treated with SDS prior to separation by SDS–PAGE [28]. Proteins were transferred overnight on to nitrocellulose and immunostained essentially as described by Towbin *et al.* [29].

RESULTS

Streptozotocin-treated animals of both sexes displayed the expected symptoms of insulin-dependent diabetes, i.e. polydipsia, hyperphagia and depressed body weight gain (results not shown). Streptozotocin caused marked increases in the plasma levels of glucose and ketone bodies in both sexes. There were not significant sex differences in the levels of these substrates in the streptozotocin-treated animals (Table 1). Similarly, no differences were evident in the basal plasma glucose and ketone concentrations between male and female control rats.

Basal total cytochrome P450 levels were lower in the female animals when compared to the male animals (Table 2). Treatment with streptozotocin increased the levels only in the female animals so that there was no significant difference between the sexes in the diabetic animals. No sex differences were observed in cytochrome b_5 and microsomal protein levels either before or after induction of diabetes. The dealkylations of pentoxyresorufin and ethoxyresorufin were induced in both sexes by the same order of magnitude (Table 2). In contrast the N-demethylation of ethylmorphine was induced only in the male animals. Treatment with streptozotocin enhanced the hydroxylations of lauric acid and *p*-nitrophenol in both sexes but the effect was more pronounced in the male animals. Immunoblot analysis of solubilized microsomes, being loaded on the basis of protein, using polyclonal antibodies showed increases in the apoprotein levels of CYP1A, CYP2B and CYP2E in both sexes, even when considering the lower levels of total cytochrome P450 in the control females (Fig. 1). When antibodies to the P4504A family were used, increases were only evident in the male animals. Finally, when the microsomes were probed with antibodies against the CYP3A family, an increase in apoprotein level was evident in the male animals (Fig. 1). An increase in CYP3A apoprotein is also detectable in the females, but this small increase is most likely to reflect, at least partly, the higher levels of total cytochrome P450 in the female diabetics when compared to the control females.

When enzyme activities are expressed per nmole of total cytochrome P450, to account for the different haemoprotein levels in the animal groups, the dealkylation of ethoxyresorufin was induced to a similar extent in both sexes (Fig. 2). The oxidation of *p*-nitrophenol and dealkylation of

Table 1. Plasma glucose and ketone body concentrations in male and female streptozotocin-treated rats

Group	Glucose (mM)	3-Hydroxybutyrate + acetoacetate (nM)
Control males	6.8 ± 0.24	0.45 ± 0.01
Streptozotocin-treated males	30.4 ± 5.0*	1.40 ± 0.11†
Control females	6.5 ± 0.54	0.51 ± 0.03
Streptozotocin-treated females	33.0 ± 7.4*	1.21 ± 0.19*

Results are presented as means ± SEM for five animals.
* $P < 0.01$; †$P < 0.001$, when compared to respective controls.

Table 2. Hepatic mixed-function oxidases in male and female streptozotocin-treated rats

Parameter	Control males	Streptozotocin-treated males	Control females	Streptozotocin-treated females
Ethoxyresorufin *O*-deethylase (pmol/min/mg protein)	10.4 ± 2.5	24.6 ± 2.5†	8.7 ± 2.0	31.4 ± 9.1*
Pentoxyresorufin *O*-depentylase (pmol/min/mg protein)	1.9 ± 0.3	10.4 ± 1.0‡	1.5 ± 0.3	7.8 ± 1.4†
Ethylmorphine *N*-demethylase (nmol/min/mg protein)	13.2 ± 0.18	28.6 ± 1.0‡	12.5 ± 0.1	13.4 ± 0.2
p-Nitrophenol hydroxylase (nmol/min/mg protein)	0.50 ± 0.10	2.84 ± 0.30†	0.58 ± 0.04	1.46 ± 0.30†
Lauric acid hydroxylase (nmol/min/mg protein)	0.99 ± 0.2	3.36 ± 0.18†	1.10 ± 0.12	2.05 ± 0.10‡
Total cytochromes P450 (nmol/mg protein)	0.55 ± 0.03	0.62 ± 0.05	0.32 ± 0.04§	0.54 ± 0.08*
Cytochrome b_5 (nmol/mg protein)	0.68 ± 0.03	0.75 ± 0.04	0.67 ± 0.07	0.90 ± 0.10
Microsomal protein (g/liver)	42.3 ± 2.5	42.6 ± 3.8	40.0 ± 4.4	35.4 ± 1.6

Results are presented as means ± SEM for five animals.
* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$, when compared to respective controls.
§ $P < 0.01$, when compared to control males.

pentoxyresorufin were induced more extensively in the male rats, whereas the N-demethylation of ethylmorphine and lauric acid hydroxylation were induced by STZ treatment only in the male animals (Fig. 2).

DISCUSSION

Two mechanisms could be responsible for the sex differences in the diabetes-induced changes in drug metabolism in rats reported by many workers [12–14]: (a) different susceptibility of the sexes to the diabetogenic properties of streptozotocin and/or (b) sex-specific differences in the modulation of cytochrome P450 proteins by insulin-dependent diabetes. The first possible mechanism can be discarded since treatment with streptozotocin elicited the same magnitude of increase in plasma glucose levels in both sexes, in agreement with previous studies [30]. Moreover, the degree of ketosis as exemplified by the plasma levels of 3-hydroxybutyrate plus acetoacetate was similar in both sexes.

The O-deethylation of ethoxyresorufin is extensively used as a diagnostic substrate for the CYP1 proteins [31]. Induction of insulin-dependent diabetes by treatment with streptozotocin enhanced this activity in the male rats, as we have reported previously [6, 8]. The same treatment given to female rats produced a similar change to those seen in the male animals. These findings were further confirmed by immunoblot analysis, employing polyclonal antibodies to the CYP1 family, showing similar increases in the apoproteins levels. Furthermore, in both sexes induction of diabetes enhanced selectively the A2 protein of the CYP1 family. The diabetes-induced changes in the CYP1 proteins appear to be mediated by the hyperketonaemia that accompanies diabetes. When animals were rendered hyperketonaemic by the dietary administration of medium chain triacyglycerols, similar changes in the CYP1 family were observed as in streptozotocin-induced diabetes [8, 32]. A determining role of ketones is also supported by the fact that administration of acetone to rats enhanced the apoprotein levels and activities of these two proteins [33]. Since the severity of ketosis is the same in both sexes, the finding that the extent of the diabetes-inducing changes in this family is similar is not surprising.

Ethylmorphine *N*-demethylase, an activity associated with the CYP3A family [34], was induced only in male animals following treatment with streptozotocin, and this is supported by the immunoblot studies where increased apoprotein levels are evident only in male rats. Although a small increase in apoprotein levels may be detectable in streptozotocin-induced female rats when compared to control, we believe this partly reflects the higher total cytochrome P450 levels in the treated animals. The CYP3A family is induced by large molecular mass compounds and therefore ketone bodies are unlikely to be responsible for the diabetes-induced changes in male rats. Indeed, male rats rendered hyperketonaemic by dietary manipulation did not exhibit the higher levels of CYP3A activity, when compared to controls, as observed in diabetic animals [7]. Moreover, treatment of male rats with acetone, 3-hydroxybutyrate or the ketone precursor 1,3-butanediol failed to influence this activity [33]. The hepatic levels of the CYP3A family are strictly regulated by the pituitary, through growth hormone. Low levels of growth hormone have been associated with increased expression of the CYP3A protein in rat liver [35]. Insulin-dependent diabetes is characterized by low circulating levels of growth hormone, a consequence of impaired release [36, 37] and it is likely that this pathological effect triggers the synthesis of P4503A proteins [7]. However, in

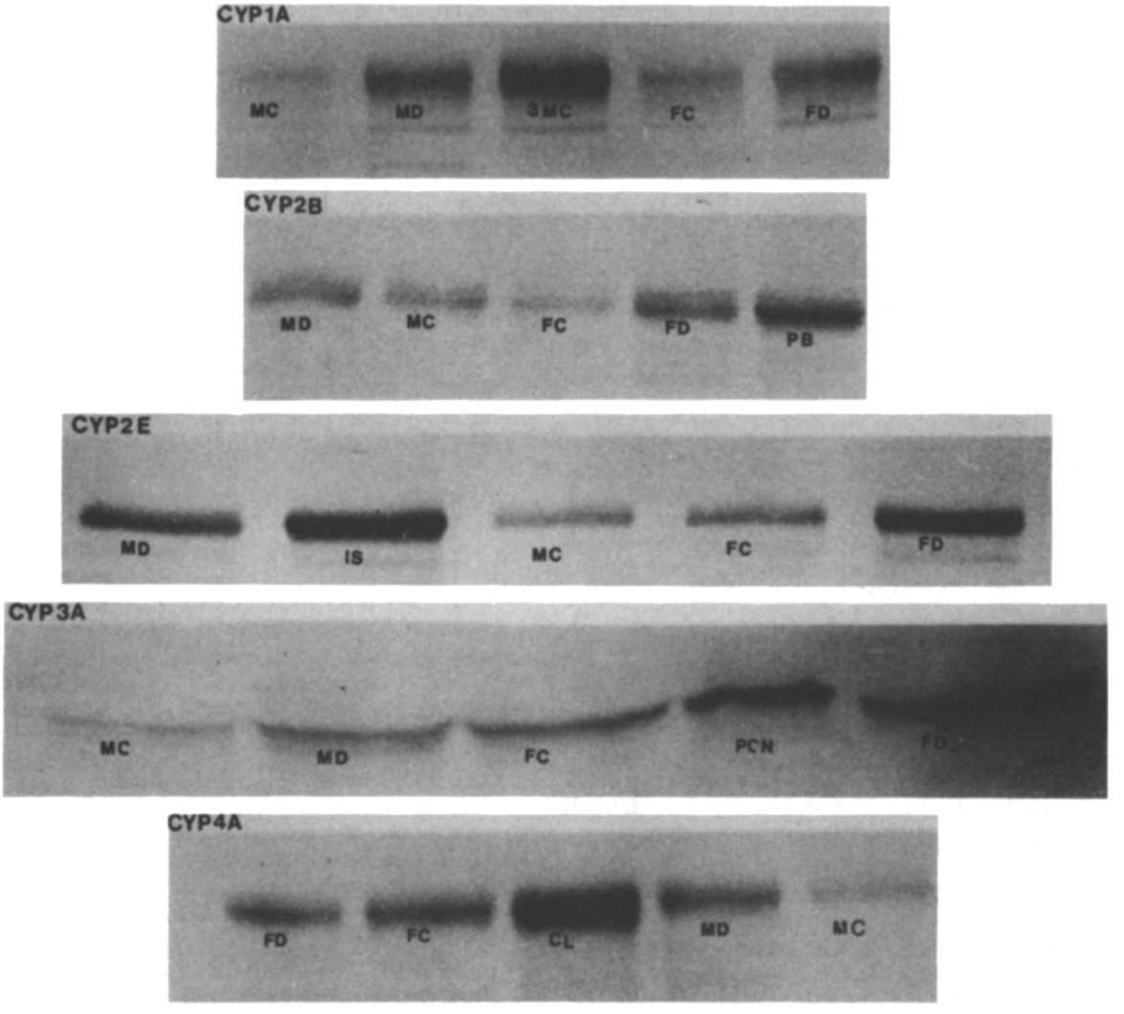

Fig. 1. Effect of streptozotocin on hepatic microsomal cytochrome P450 proteins in the male and female rat determined using immunoblots. Microsomal proteins were resolved by electrophoresis in a 10% (w/v) SDS-polyacrylamide gel and transferred to nitrocellulose. In all cases 20 μg of solubilized microsomal protein were loaded except for the positive controls when only 10 μg of protein were loaded. The following primary antibody dilutions were used: sheep anti-CYP1A, 1:10,000; rabbit anti-CYP2B, 1:2000; sheep anti-CYP-2E, 1:5000; rabbit anti-CYP3A, 1:2000 and sheep anti-CYP4A, 1:5000. The dilutions for the secondary antibodies were: peroxidase-linked donkey anti-sheep IgG, 1:2000 and peroxidase-linked goat anti-rabbit IgG, 1:2000. MC, male control; FC, female control; MD, male diabetic; FD, female diabetic; 3MC, 3- methylcholanthrene-treated; PB, phenobarbital-treated; IS, isoniazid-treated; PCN, PCN-treated and CL, clofibrate-treated.

contrast to male rats, induction of diabetes by treatment of female rats with streptozotocin is not associated with changes in the pattern of growth hormone secretion [38], explaining the lack of change in the level of CYP3A activity as exemplified by the N-demethylation of ethylmorphine.

The CYP4A is unique among the inducible forms of cytochrome P450 in that it does not metabolize the inducing substrate or any other xenobiotic, but appears to be involved exclusively in the metabolism of endogenous substrates, such as fatty acids and eicosanoids [39]. When expressed in terms of total cytochrome P450, diabetes caused an increase in lauric acid hydroxylase, an enzyme specific to the CYP4A family, only in male rats, and this was supported by immunoblot analysis of the apoprotein levels. Previous studies revealed that dietary administration of medium chain triglycerides enhances lauric acid hydroxylation and increases CYP4A apoprotein levels [7]. As this treatment induces hyperketonaemia, it was considered likely that, as in the case of the CYP1 family, the circulating high ketone levels acted as inducers of the CYP4A family. However, this mechanism appears unlikely since treatment of male rats with acetone or other physiological ketones failed to induce CYP4A activity [33]. These observations indicate that the triglycerides are responsible, directly or indirectly,

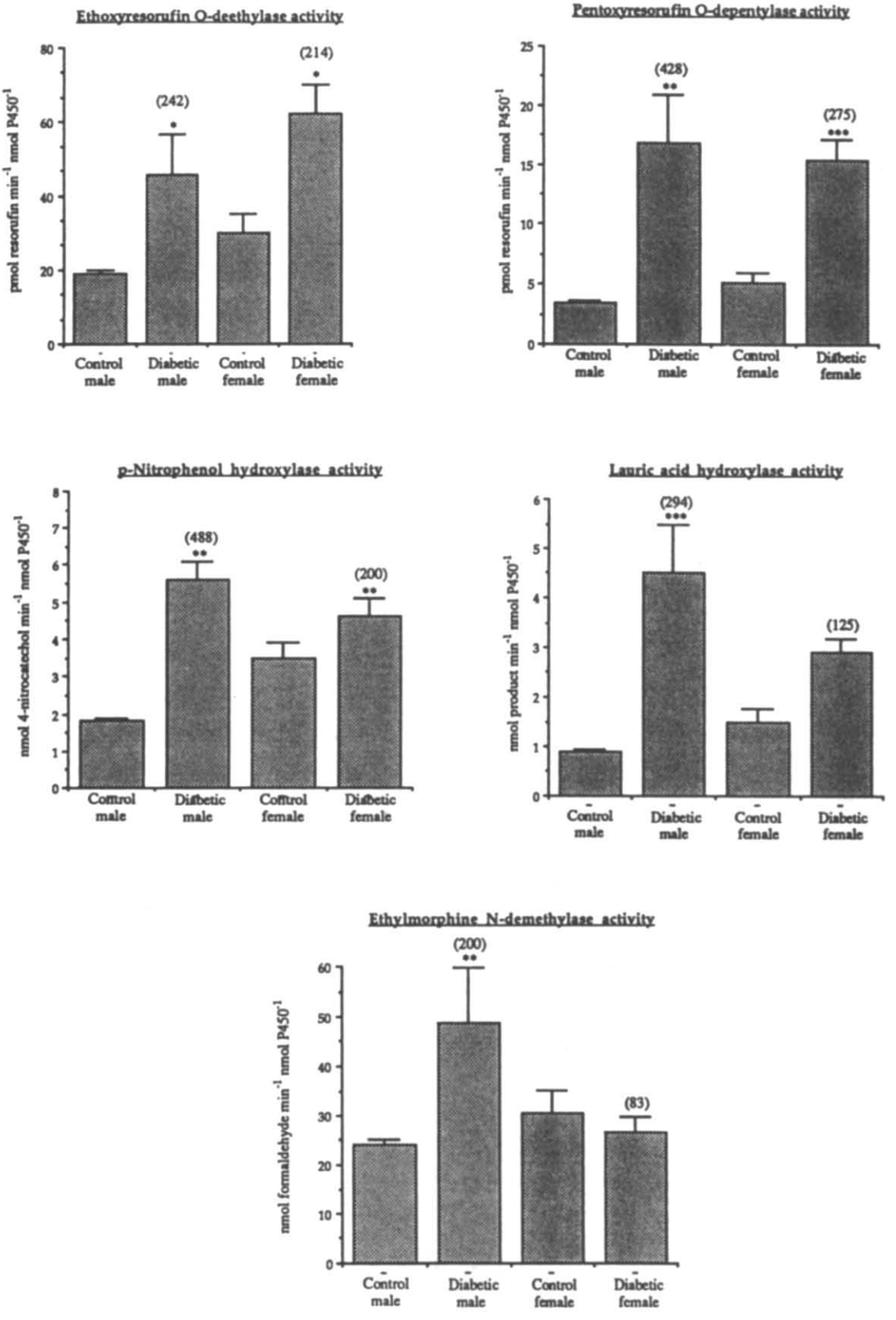

Fig. 2. Microsomal cytochrome P450 activities expressed per nmole of P450 for male and female control and diabetic rats. Results are presented as means ± SEM for five animals. Numbers in parentheses indicate the % of control activities. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

for the increase in CYP4A activity through a mechanism independent of hyperketonaemia. The hyperlipidaemia that accompanies diabetes may play a significant role, therefore, in the induction of the CYP4A proteins. Compounds that increase the plasma levels of triglycerides have been reported as capable of inducing the CYP4A family [40]. The mechanism for this sex difference in the diabetes-induced CYP4A activity is not clear. One can speculate that it reflects different degrees of hyperlipidaemia in the two sexes in the rat. Indeed, in clinical studies, sex differences in the effects of insulin-dependent diabetes on plasma cholesterol, triglyceride and lipoprotein levels have been documented [41].

Finally, streptozotocin-induced diabetes enhanced *p*-nitrophenol hydroxylase and pentoxyresorufin *O*-dealkylase, diagnostic probes for the E and B subfamilies of the CYP2 family [20, 42], in both sexes but the effect was clearly more pronounced in the male animals. Hyperketonaemia is, at least partly, responsible for the increase in these subfamilies since: (a) correlations have been established between the plasma ketone levels on one hand and CYP2E levels and activity [9]; (b) hyperketonaemia induced by the administration of medium chain triglycerides increased the levels of CYP2B and CYP2E proteins [31] and (c) treatment of animals with acetone elevated the hepatic levels and enhanced the activity of CYP2B and CYP2E proteins [33, 43, 44]. As the degree of hyperketonaemia induced by streptozotocin is the same in both sexes, a similar increase in CYP2B and CYP2E activities would be expected, contrary to the present findings. However, it has been reported recently that a decrease in growth hormone levels may also trigger the synthesis of proteins belonging to these subfamilies in the rat [45]. It therefore appears likely that in male diabetic rats two mechanisms contribute to the increased CYP2B and CYP2E activities: a reduction in the plasma levels of growth hormone and the high plasma levels of the ketones; whereas in female animals only the latter is operative, thus explaining the observed sex difference.

In summary, the present work demonstrates that: (a) treatment of male and female rats with streptozotocin induces insulin-dependent diabetes of similar severity; (b) the sex differences in the diabetes-induced changes in cytochromes P450 are specific to certain families and (c) the difference may be related to sex differences in growth hormone levels and degree of hyperlipidaemia in the diabetic animals.

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REFERENCES

1. Guengerich FP, Roles of cytochrome P-450 enzymes in chemical carcinogenesis and cancer chemotherapy. *Cancer Res* **48**: 2946–2954, 1988.
2. Ioannides C and Parke DV, The cytochrome P450I gene family of microsomal haemoproteins and their role in the metabolic activation of chemicals. *Drug Metab Rev* **22**: 1–85, 1990.
3. Ryan DE and Levin W, Purification and characterization of hepatic cytochrome P-450. *Pharmacol Ther* **45**: 153–239, 1990.
4. Gonzalez FJ, Molecular genetics of the P-450 superfamily. *Pharmacol Ther* **45**: 1–38, 1990.
5. Gibson GG, Comparative aspects of mammalian cytochrome P450IV gene family. *Xenobiotica* **19**: 1123–1148, 1989.
6. Ioannides C, Bass SL, Ayrton AD, Trinick J, Walker R and Flatt PR, Streptozotocin-induced diabetes modulating the metabolic activation of chemical carcinogens. *Chem Biol Interact* **68**: 189–202, 1988.
7. Barnett CR, Gibson GG, Wolf CR, Flatt PR and Ioannides C, Induction of cytochrome P-450III and P-450IV family proteins in streptozotocin-induced diabetes. *Biochem J* **286**: 765–769, 1990.
8. Barnett CR, Flatt PR and Ioannides C, Induction of hepatic microsomal P-450I and IIB proteins by hyperketonaemia. *Biochem Pharmacol* **40**: 393–397, 1990.
9. Bellward GD, Chang T, Rodrigues B, McNeill JH, Maines S, Ryan DE, Levin W and Thomas PE, Hepatic cytochrome P-450j induction in the spontaneously diabetic BB rat. *Mol Pharmacol* **33**: 140–143, 1988.
10. Barnett CR, Wilson J, Wolf CR, Flatt CR, Flatt PR and Ioannides C, Hyperinsulinaemia causes a preferential increase in hepatic P4501A2 activity. *Biochem Pharmacol* **43**: 1255–1261, 1992.
11. Ioannides C, Metabolism and toxicity of chemicals in diabetes. *Acta Pharmaceut Jugoslav* **40**: 423–429, 1990.
12. Kato R, Onoda IK and Takanaka A, Species differences in drug metabolism by liver microsomes in alloxan diabetic of fasted animals. *Jpn J Pharmacol* **20**: 554–561, 1970.
13. Reinke LA, Stohs SJ and Rosenberg H, Increased aryl hydrocarbon hydroxylase activity in hepatic microsomes from streptozotocin diabetic female rats. *Xenobotica* **8**: 769–778, 1979.
14. Past MR and Cook DE, Catalytic activities of cytochrome P-450 from female rat liver: correlation with sex differences in drug metabolism in diabetic liver. *Res Commun Chem Pathol Pharmacol* **40**: 379–390, 1983.
15. Rodrigues AD, Gibson GG, Ioannides C and Parke DV, Interactions of imidazole antifungal agents with purified cytochrome P-450 proteins. *Biochem Pharmacol* **36**: 4277–4281, 1987.
16. Wilson AM, Christou M and Jefcoate CR, Differential expression and function of three closely related phenobarbital-inducible cytochrome P-450 isoenzymes in untreated liver. *Arch Biochem Biophys* **256**: 407–420, 1987.
17. Smith JN, Ayrton AD, Chown J, Lewis DFV and Ioannides C, Feprazone: an inducer of the P450IIB family of proteins in the rat. *J Biochem Toxicol* **5**: 9–12, 1990.
18. Ioannides C and Parke DV, Mechanism of induction of hepatic microsomal drug metabolising enzymes by a series of barbiturates. *J Pharm Pharmacol* **27**: 739–746, 1975.
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, Wolf T and Guengerich FP, Dealkylation of pentoxyresorufin: a rapid and sensitive assay for measuring induction by phenobarbital and other xenobiotics in the rat. *Arch Biochem Biophys* **238**: 43–48, 1985.
21. Reinke LA and Moyer MJ, *p*-Nitrophenol hydroxy-

lation: a microsomal oxidation which is highly inducible by ethanol. *Drug Metab Dispos* **13**: 548–552, 1985.
22. Lu AYH, Kuntzman R, West S, Jacobson M and Conney AH, Reconstituted liver microsomal enzyme system that hydroxylates drugs, other foreign compounds and endogenous substrates. *J Biol Chem* **247**: 1727–1733, 1972.
23. Omura T and Sato R, The carbon monoxide pigment of liver microsomes. I. Evidence for its haemoprotein nature. *J Biol Chem* **239**: 2370–2378, 1964.
24. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
25. Parker G and Orton T, Induction by oxyisobutyrates of hepatic and kidney microsomal cytochrome P-450 with specificity towards hydroxylation of fatty-acids. In: *Biochemistry, Biophysics and Regulation of Cytochrome P-450* (Eds. Gustaffson JA, Mode A and Rafter J), pp. 373–377. Elsevier/North Holland, Biomedical Press, Amsterdam, 1980.
26. Stevens J, Determination of glucose by an automatic analyser. *Clin Chim Acta* **32**: 199–201, 1971.
27. McGarry JD, Guest MJ and Foster DW, Ketone body metabolism in the ketosis of starvation and alloxan diabetes. *J Biol Chem* **245**: 4382–4390, 1970.
28. Laemmli UK, Cleavage of structural proteins during the assembly of the head bacteriophage T_4. *Nature* **227**: 680–685, 1979.
29. Towbin H, Staehelin T and Gordon J, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* **76**: 4350–4355, 1979.
30. Skett P, Sex-dependent effect of streptozotocin-induced diabetes mellitus on hepatic steroid metabolism in the rat. *Acta Endocrinol* **111**: 217–221, 1986.
31. Phillipson CE, Godden PMM, Lum PY, Ioannides C and Parke DV, Determination of cytochrome P-448 activity in biological tissues. *Biochem J* **221**: 81–88, 1984.
32. Barnett CR, Flatt PR and Ioannides C, Role of ketone bodies in the diabetes-induced changes in hepatic mixed-function oxidase activities. *Biochim Biophys Acta* **967**: 250–254, 1988.
33. Barnett CR, Petrides L, Wilson J, Flatt PR and Ioannides C, Induction of the rat hepatic mixed-function oxidases by acetone and other physiological ketones: their role in the diabetes-induced changes in cytochrome P-450 proteins. *Xenobiotica*, in press.
34. Wrighton SA, Maurel P, Scheutz EG, Watkins PB, Young B and Guzelian PS, Identification of the cytochrome P-450 induced by macrolide antibiotics in the rat liver as the glucocorticoid responsive cytochrome P-450p. *Biochemistry* **24**: 2171–2178, 1985.
35. Lemoine A, Marie S and Cresteil T, Expression of cytochrome P-450 isozymes in the liver of hypophysectomised rats. *Eur J Biochem* **177**: 597–604, 1988.
36. Serri O and Brazeau B, Growth hormone responsiveness *in vivo* and *in vitro* to growth hormone releasing factor in the spontaneously diabetic BB Wistar rat. *Neuroendocrinology* **46**: 162–166, 1987.
37. Tannenbaum GS, Growth hormone secretory dynamics in streptozotocin diabetes: evidence of a role for endogenous circulating somatostatin. *Endocrinology* **108**, 76–82, 1981.
38. Thummel KE and Schenkman JB, Effects of testosterone and growth hormone treatment in the diabetic rat. *Mol Pharmacol* **37**: 119–129, 1990.
39. Tamburini P, Masson HA, Bains SK, Makowski RJ, Morris B and Gibson GG, Multiple forms of hepatic cytochrome P-450: purification, characterisation and comparison of a novel clofibrate-induced isozyme with other major forms of cytochrome P-450. *Eur J Biochem* **139**: 235–246, 1984.
40. Hardwick JP, Song BY, Huberman E and Gonzalez FJ, Isolation and complementary DNA sequence and regulation of rat hepatic lauric acid ω-hydroxylase (cytochrome $P\text{-}450_{LA\omega}$). *J Biol Chem* **262**: 801–810, 1987.
41. Walden CE, Knopp RH, Wahl PW, Beach KW and Strandness JR, Sex differences in the effect of diabetes mellitus on lipoprotein, triglyceride and cholesterol concentrations. *N Engl J Med* **311**: 953–959, 1984.
42. Koop DR, Laethem CL and Tierney DJ, The utility of *p*-nitrophenol hydroxylation in P450IIE1 analysis. *Drug Metab Rev* **20**: 541–551, 1989.
43. Koop DR, Crump BL, Nordblom GD and Coon MJ, Immunochemical evidence for induction of the alcohol-oxidising cytochrome P-450 of rabbit liver microsomes by diverse agents: ethanol, imidazole, trichloroethylene, acetone, pyrazole and isoniazid. *Proc Natl Acad Sci USA* **82**: 4065–4069, 1985.
44. Song BJ, Gelboin HV, Park S-S, Yang CS and Gonzalez FJ, Complementary DNA and protein sequences of ethanol-inducible rat and human cytochrome P-450s. *J Biol Chem* **261**: 16689–16697, 1986.
45. Yamazoe Y, Murayama N, Shimada M, Yamauchi K and Kato R, Cytochrome P-450 in livers of diabetic rats: regulation by growth hormone and insulin. *Arch Biochem Biophys* **268**: 567–575, 1989.