Immunochemical quantification of cytochrome *P*450IA and IIB subfamilies in the livers of metyrapone-treated rats

Relevance to the ability of metyrapone to prevent the loss of cytochrome *P*-450 in rat hepatocyte culture

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Polyclonal antibodies to the major β -naphthoflavone (BNF)-inducible form of cytochrome *P*-450 (*P*450IA) and to the major phenobarbitone (PB)-inducible form (*P*450IIB) have been used to quantify the contribution of these subfamilies to the total amount of cytochrome *P*-450 in rat livers and rat hepatocyte cultures treated with PB, BNF and metyrapone for 24 and 72 h. The *P*450IA and IIB subfamilies were not detectable (less than 5 pmol/mg of microsomal protein) in the livers of control rats, but administration of BNF resulted in the *P*450IA subfamily comprising more than 80 % of the total hepatic cytochrome *P*-450. Administration of PB and metyrapone to rats did not elevate the level of this subfamily but elevated the levels of the *P*450IIB subfamily to 60 % and 30 % respectively of the total. Thus metyrapone is a 'PB-like' inducer. However, in contrast with their effects *in vivo*, treatment with PB and metyrapone of rat hepatocytes did not elevate the proportion of the *P*450IIB subfamily relative to that in untreated cells but rather, like BNF, increased the *P*450IA subfamily. This would account for the ability of metyrapone to produce in hepatocyte culture, like BNF, a pronounced induction of ethoxyresorufin *O*-de-ethylase activity, but it does not account for why of all inducers studied only metyrapone can maintain the total cytochrome *P*-450 content of cultured hepatocytes, or the activity of ethylmorphine *N*-demethylase. This activity is generally considered to be associated with the *P*450IIB subfamily, but the lack of effect of metyrapone on this subfamily in hepatocyte culture must suggest that metyrapone is able to prevent the loss of the total amount of the cytochrome by increasing the expression of other cytochromes *P*-450.

INTRODUCTION

Metyrapone [2-methyl-1,2-di-(3-pyridyl)-1-propanone] is a commonly used inhibitor of cytochrome *P*-450-mediated xenobiotic metabolism *in vitro* and *in vivo* [1]. However, treatment of rats with a single dose of 100 mg of metyrapone/kg body weight results in a pronounced induction of hepatic microsomal cytochrome *P*-450 content within 24 h of administration [2]. Indeed, treatment of rats with metyrapone for 24 h has been found to increase hepatic cytochrome *P*-450 content to the same extent as equivalent doses of the classical inducer phenobarbitone (PB). Furthermore, based on the range of *P*-450-mediated enzyme activities increased, metyrapone has been proposed to be a PB rather than a polycyclic hydrocarbon (β -naphthoflavone; BNF) type of inducer [2]. However, when rat hepatocytes were cultured with metyrapone, not only was the spontaneous loss of cytochrome *P*-450 prevented, but the activity of drug-metabolizing enzymes generally considered to be associated with the PB-inducible cytochrome *P*450IIB subfamily was maintained for 72 h at the same level as found in the intact liver from which the cultures were prepared. In contrast, those activities associated with the polycyclic-hydrocarbon-inducible *P*450IA subfamily were markedly induced [3,4]. In order to rationalize these observations and to document further the response of cultured hepatocytes to inducers, the present work employing polyclonal antibodies to quantify the effect of treatment with metyrapone and the archetypal inducers upon their content of *P*450IA and *P*450IIB subfamilies was undertaken.

MATERIALS AND METHODS

Materials

PB sodium, dimethyl formamide, glycerol, acrylamide, bis-acrylamide and dithiothreitol were obtained from BDH, Poole, Dorset, U.K. Sodium pentobarbitone (Sagatal injection) was from May and Baker Ltd., Dagenham, Essex, U.K. BNF, metyrapone, butylated hydroxytoluene (BHT), phenylmethane-sulphonyl fluoride, BSA, insulin, cortisol 21-sodium succinate, FMN, Tween 20, prestained *M*_r markers and Freund's incomplete and complete adjuvants were purchased from Sigma Chemical Co., Poole, Dorset, U.K. Collagenase H was from Boehringer, Lewes, East Sussex, U.K. Williams medium E, foetal calf serum and gentamycin were from Flow Laboratories, Irvine, Scotland, U.K. Schleicher and Schuell nitrocellulose membrane was purchased from Anderman and Co., Kingston, Surrey, U.K. Donkey anti-rabbit Ig (¹²⁵I-labelled species-specific whole antibody) was purchased from Amersham International, Aylesbury, Bucks., U.K. Goat anti-rabbit IgG (heavy and light chains) conjugated with horseradish peroxidase was purchased from Bio-Rad Laboratories, Watford, Herts., U.K. All other chemicals were of the highest purity available from commercial sources.

Animals

All experiments were performed on adult male CD rats, supplied by Charles River Ltd., Margate, Kent, U.K., which weighed 200–250 g at the start of the experiments.

Abbreviations used: PB, phenobarbitone; BNF, β -naphthoflavone; BHT, butylated hydroxytoluene; TBS, Tris-buffered saline.

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Animal treatment and microsome preparation

Rats were given either PB (100 mg/kg body weight) or metyrapone (100 mg/kg body weight), both dissolved in 0.15 M-NaCl, or BNF (80 mg/kg body weight) dissolved in corn oil, by intraperitoneal injection. The inducers were administered once only or daily for 3 consecutive days. Control animals received the injection vehicle (4 ml/kg body weight). At 24 or 72 h after the first dose of inducer, the animals were killed by decapitation, their livers were removed and a 20% (w/v) homogenate was prepared using an Ultra-Turrax type 18/10 blender in ice-cold 0.1 M-Tris/acetate buffer, pH 7.4, containing 0.1 M-KCl, 1 mM-EDTA and 23 μ M-BHT. The microsomal fraction was prepared by differential centrifugation as described previously [2]. The resultant microsomal pellets were resuspended at a concentration of 20 mg of microsomal protein/ml in ice-cold 10 mM-potassium phosphate buffer, pH 7.25, containing 1 mM-EDTA and 20% (v/v) glycerol; the suspensions were then frozen and stored in liquid N₂.

Purification of cytochromes P-450 and preparation of antibodies

Cytochrome P450IA and IIB subfamilies were purified from the livers of groups of 12 male rats which had been treated with BNF or PB for 3 days, using the method described by Guengerich *et al.* [5]. The exact procedure employed in this laboratory has been described in detail [6]. Antibodies to these isoenzymes were raised in half-lop rabbits by injecting 1 mg of the isoenzyme preparation emulsified in 800 μ l of Freund's complete adjuvant at two intramuscular, one subcutaneous and one intraperitoneal sites. Three weeks later, a booster of 1 mg of isoenzyme emulsified in Freund's incomplete adjuvant was administered, and serum was prepared from the rabbits 1 week later.

Hepatocyte isolation and culture

Hepatocytes were isolated by perfusion of the livers of rats, anaesthetized with sodium pentobarbitone, with 0.025% (w/v) collagenase H, and were maintained as primary monolayer cultures for up to 3 days as described previously [7]. The culture medium was Williams medium E containing 5% (v/v) foetal calf serum, 1 μ M-insulin, 0.1 mM-cortisol 21-sodium succinate and 50 μ g of gentamycin/ml. Metyrapone and PB were dissolved directly in the culture medium to give final concentrations of 0.5 mM and 2 mM respectively, whereas BNF was dissolved in dimethyl formamide and added to the culture medium to give final concentrations of 10 μ M-BNF and 0.25% (v/v) dimethyl formamide. The cells were incubated at 37 °C under a humidified atmosphere of 5% (v/v) CO₂ in air and the culture medium was changed daily. The cells from twelve 150 mm diameter Petri dishes for each treatment regime were scraped off the dishes and homogenized, by 3 $\times$ 10 s bursts of an Ultra-Turrax blender, in 25 ml of ice-cold 0.1 M-Tris/acetate buffer, pH 7.4, containing 0.1 M-KCl, 1 mM-EDTA and 23 μ M-BHT. The microsomal fraction was then prepared and stored as described above for intact liver.

Immunoblotting and quantification of rat hepatic cytochromes P-450

The microsomal fractions from intact liver and cultured hepatocytes were diluted to 2 mg of protein/ml in ice-cold 0.1 M-potassium phosphate buffer (pH 7.25) containing 20% (v/v), glycerol, 1 mM-dithiothreitol, 1 mM-EDTA, 2 μ M-FMN, 23 μ M-BHT and 0.4 mM-phenylmethanesulphonyl fluoride. While this microsomal suspension was stirred at 4 °C, a 20% (w/v) solution of sodium cholate was slowly added, dropwise, from a Pasteur pipette, to give a final concentration of 0.7% (w/v) sodium cholate. When the addition was complete, the samples continued to be stirred at 4 °C for a further 15 min and were then centrifuged

at 4 °C at 100 000 g for 60 min. The supernatants were frozen and stored in liquid N₂.

Samples (15 μ l; approx. 20 μ g of protein) of each microsomal preparation were loaded for SDS/PAGE on to a gel comprising 3.75% (w/v) acrylamide as the stacking gel and 10% (w/v) acrylamide as the resolving gel; both gels were prepared from a stock solution of 30% (w/v) acrylamide containing 0.4% bisacrylamide. These gels contained 0.1% (w/v) SDS. After electrophoresis (60 mA for 1.5 h), the proteins were transferred from the gels to nitrocellulose sheets according to the method of Towbin *et al.* [8], except that a current of 0.3 A applied for 2 h at 20 °C was used, as this was found to result in the complete transfer of the proteins. The Western blots were washed in Tris-buffered saline (TBS; 0.2 M-NaCl/20 mM-Tris/HCl, pH 7.4) for 10 min. To block non-specific binding of the antibodies to the nitrocellulose, the blots were then treated for 16 h at 4 °C in TBS containing 3% (w/v) BSA and 0.3% (v/v) Tween 20. The blots were then incubated at room temperature (20 $\pm$ 3 °C) for 1 h in TBS containing 0.3% BSA, 0.05% (v/v) Tween 20 and a 1:100 dilution of the rabbit polyclonal antibody to the specific form of cytochrome P-450 being measured. The blots were then washed (3 $\times$ 15 min) in TBS containing 0.05% (v/v) Tween 20 before incubation with either a 1:3000 dilution of goat anti-rabbit IgG (H + L) conjugated with horseradish peroxidase or 5 μ Ci of ¹²⁵I-labelled anti-rabbit Ig (specific radioactivity 5–20 μ Ci/ μ g of antibody protein) in TBS containing 0.3% BSA and 0.05% Tween 20 for 1 h at room temperature. The blots were finally washed for 3 $\times$ 15 min in TBS containing 0.05% (v/v) Tween 20 before revealing immunoreactive material by incubation with 0.02% (v/v) H₂O₂ and 0.04% (w/v) 4-chloro-1-naphthol (see Fig. 1) or autoradiography employing Fuji X-ray film (see Fig. 2). After development of the autoradiographs, the density of the bands corresponding to the cytochrome P450IA and IIB subfamilies was determined using an LKB Ultro-Scan XL laser densitometer employing LKB 2400 Gel-Scan XL software. The amounts of the specific form of cytochrome P-450 were quantified by reference to a standard curve (0.1–1.0 pmol of P-450) prepared from purified P450IA and IIB which were run on the same gel. The values from the immunochemical quantification of these isoenzymes are expressed as a percentage of the total cytochrome P-450 content in the sample, as determined by its reduced-CO versus reduced difference spectrum [2].

Protein determination

The protein content of all samples was determined by the method of Lowry *et al.* [9], employing BSA as a standard.

Statistics

Statistical comparisons were carried out using Student's *t* test (two-tailed) and a value of *P* exceeding the 5% level of confidence was deemed significant.

RESULTS AND DISCUSSION

The P450IA preparation used in this work had a specific content of 16.5 nmol of cytochrome P-450/mg of protein and migrated on SDS/PAGE as two species with *M_r* values of 52 500 and 54 000, which are compatible with the *M_r* values of P450IA1 and P450IA2 [10]. The P450IIB preparation had a specific content of 17.9 nmol of cytochrome/mg of protein and migrated on SDS/PAGE as one species with an *M_r* of 51 500, in agreement with the *M_r* of both P450IIB1 and P450IIB2 which have a 97% sequence identity [10]. Fig. 1 shows Western blots of purified P450IA (lanes 7), purified P450IIB (lanes 2) and hepatic microsomes prepared from control (lanes 3) and PB- (lanes 4), BNF- (lanes 5) and metyrapone-treated (lanes 6) rats which have been

Fig. 1. Immunoblots of purified rat hepatic *P450IA* and *P450IIB* and rat liver microsomes probed with rabbit antisera to *P450IA* (a) and *P450IIB* (b)

Visualization of cytochromes *P-450* is with goat anti-rabbit IgG conjugated to horseradish peroxidase (see the Materials and methods section). Lanes 1, M_r markers (α_2 -macroglobulin, 180000; β -galactosidase, 116000; fructose-6-phosphate kinase, 84000; pyruvate kinase, 58000; fumarase, 48500; lactate dehydrogenase, 36500; triosephosphate isomerase, 26600). Lanes 2, 20 pmol of purified *P450IIB*. Lanes 3, 4, 5 and 6, hepatic microsomes from control, PB-, BNF- and metyrapone-treated rats. Lanes 7, 20 pmol of purified *P450IA*.

probed with antibody to the *P450IA* (Fig. 1a) and *P450IIB* (Fig. 1b) subfamilies. It is evident that the antibody raised against the *P450IA* subfamily does not cross-react with the *P450IIB* subfamily and vice-versa. Incubation of Western blots with

Fig. 2. Autoradiographs of purified *P450IA* (a) and purified *P450IIB* (b)

The cytochromes were electrophoresed by SDS/PAGE, blotted and incubated with the corresponding rabbit antibody followed by incubation with ^{125}I -labelled goat anti-rabbit IgG as described in the Materials and methods section. Autoradiography was performed at -70°C for 2 weeks before development of the X-ray film and quantification by laser densitometry. The amount of each subfamily in microsomal extracts of rat livers and cultured hepatocytes was quantified by carrying doubling dilutions of the extracts through the procedure, and the density falling closest to the 0.5 pmol standard was used for quantification. This is presented in Tables 1 and 2. Amounts of purified cytochromes *P-450* used were: lanes 1, 0.1 pmol; lanes 2, 0.25 pmol; lanes 3, 0.5 pmol; lanes 4, 1 pmol; lanes 5, 2 pmol; lanes 6, 4 pmol.

preimmune sera did not result in the appearance of immunoreactive material (results not shown). However, both antibodies did reveal a minor immunoreactive species of a lower M_r than purified *P450IA* and *P450IIB* in hepatic microsomes prepared from untreated as well as inducer-treated rats. The identity of this species is unknown at present, as it is not evident in the purified antigens and its staining intensity was not affected by the inducers studied (Figs. 1a and 1b). Nevertheless, Fig. 1(a) shows that the *P450IA* subfamily was only detected in hepatic microsomes from BNF-treated rats, whereas Fig. 1(b) shows that the *P450IIB* subfamily was detected only in microsomes from PB- and metyrapone-treated animals. Accordingly, the results shown in Fig. 1 confirm that the inducing effect of metyrapone administration to rats is qualitatively similar to that of PB administration, in that it increases the content of the *P450IIB* subfamily.

The antibodies were then used to quantify the effects of inducers on the hepatic content of the *P450IA* and *P450IIB* subfamilies. In order to exclude the contribution of the minor immunoreactive material present in microsomal suspensions to the final values for the *P450IA* and *IIB* subfamilies, Western

Table 1. Effect of administration of inducers to rats on their hepatic microsomal cytochrome *P-450* concentration and the relative proportions of the *P450IA* and *IIB* subfamilies

The respective inducers were administered to rats for 24 or 72 h, after which their hepatic microsomal fractions were prepared, the cytochrome *P-450* content was measured, and Western blots were performed and quantified as described in the Materials and methods section. Hepatic microsomal cytochrome *P-450* contents are presented as means $\pm$ S.D. for three individual animals. The percentages of each subfamily in hepatic microsomes from untreated, PB-treated and metyrapone-treated rats are presented as the individual values found in two animals, while BNF treatment, employed as a positive control, constitutes the result from only one animal. There were no significant differences in the values for corn-oil- or saline-treated controls, so, for brevity, only the data for saline-treated animals are presented. n.d., not detectable; less than 5 pmol/mg of microsomal protein. *Indicates significantly different ($P < 0.05$) from control values by Student's *t* test.

Inducer	Incubation period...	Cytochrome P450 concn. (nmol/mg of microsomal protein)		Subfamily content (% of total hepatic cytochrome <i>P-450</i>)			
				<i>P450IA</i>		<i>P450IIB</i>	
		24 h	72 h	24 h	72 h	24 h	72 h
None		0.98 $\pm$ 0.09	0.95 $\pm$ 0.09	n.d.	n.d.	n.d.	n.d.
BNF		1.20 $\pm$ 0.16	1.71 $\pm$ 0.21*	82	110	n.d.	15
Metyrapone		1.42 $\pm$ 0.12*	1.44 $\pm$ 0.10*	n.d.	n.d.	30; 34	28; 28
PB		1.66 $\pm$ 0.17*	2.27 $\pm$ 0.19*	n.d.	n.d.	58; 64	56; 60

Table 2. Effect of hepatocyte culture conditions upon their cytochrome *P*-450 content and the relative proportions of the *P*450IA and IIB subfamilies

Rat hepatocytes were cultured without treatment, or with 10 μ M-BNF, 0.5 mM-metyrapone or 2 mM-PB for 24 h and 72 h. Hepatocytes from twelve 150 mm diameter Petri dishes for each treatment were homogenized, the microsomal fraction was prepared, total cytochrome *P*-450 content was measured and Western blots were performed and quantified as described in the Materials and methods section. The results are presented as means $\pm$ s.d. of three individual determinations. n.d., not detectable. The initial cytochrome *P*-450 concentration of the hepatocytes prior to culture was 168 ± 20 pmol/mg of protein.

Inducer	Incubation period...	Cytochrome <i>P</i> -450 concn. (% of initial value)		Subfamily content (% of total hepatic cytochrome <i>P</i> -450)			
				<i>P</i> 450IA		<i>P</i> 450IIB	
		24 h	72 h	24 h	72 h	24 h	72 h
None		48 $\pm$ 5	23 $\pm$ 5	n.d.	n.d.	21.0 $\pm$ 1.4	n.d.
BNF		67 $\pm$ 8	31 $\pm$ 6	21.0 $\pm$ 2.9	100.7 $\pm$ 21.0	28.5 $\pm$ 11.9	n.d.
Metyrapone		95 $\pm$ 3	101 $\pm$ 6	8.8 $\pm$ 3.5	40.0 $\pm$ 10.0	14.7 $\pm$ 5.0	n.d.
PB		55 $\pm$ 6	29 $\pm$ 4	n.d.	42.3 $\pm$ 8.3	21.0 $\pm$ 1.4	n.d.

blotting followed by incubation with a second 125 I-labelled antibody and subsequent autoradiography and densitometry was preferred to other methods of quantification (such as e.l.i.s.a.). Fig. 2 shows representative standard curves prepared from *P*450IA (Fig. 2a) and *P*450IIB (Fig. 2b) subfamilies. The different densities observed with the same amount of these isoenzymes presumably reflects the differing affinities of the polyclonal antibodies employed in their quantification. However, the density of the autoradiographs was found to be proportional to the amount of each isoenzyme over the range 0.1–4 pmol. In practice, doubling dilutions of microsomal extracts were blotted and autoradiographed, and the density falling closest to the 0.5 pmol standard was used for quantification. The results of this approach are presented in Table 1, and show that administration of metyrapone to rats is not as effective as PB administration at increasing the relative proportion of the *P*450IIB subfamily. Thus, although the cytochrome *P*-450 content of microsomes prepared from the livers of PB- and metyrapone-treated rats were not significantly different 24 h after treatment, hepatic microsomes from rats treated with PB contained a much higher proportion of the hepatic *P*450IIB subfamily than in animals treated with metyrapone (Table 1). Also, the time course of the induction by PB is different from that by metyrapone in that the effects of the latter on hepatic microsomal cytochrome *P*-450 content are maximal after 24 h of treatment, whereas repeated administration of PB results in a higher microsomal *P*-450 content after 72 h of treatment (Table 1). The values for the effect of PB treatment on the *P*450IIB subfamily are in agreement with the values measured by other investigators, as is the finding that only the administration of polycyclic hydrocarbons such as BNF to rats increases the proportion of the *P*450IA subfamily [11]. The observation that there is more immunoreactive *P*450IA apoprotein than holocytochrome in BNF-treated rats may suggest that the synthesis or incorporation of the haem prosthetic group is limiting.

When hepatocytes prepared from untreated rats are cultured for 24 h, their total cytochrome *P*-450 content is halved, but, in contrast with the situation with intact liver, the proportion of the cytochrome *P*450IIB subfamily increases above the lower level of detection and comprises 21 % of the total cytochrome present in the hepatocyte (Table 2). Again in contrast with the situation observed in the livers of intact animals, treatment of hepatocyte cultures with PB or metyrapone did not increase the relative proportion of the cytochrome *P*450IIB subfamily above that found in untreated cultures. The only effect in accord with the

animal data resulted from the treatment of hepatocyte cultures with BNF, when, after 24 h and 72 h of treatment, the cytochrome *P*450IA subfamily was greatly increased, and again there was more immunoreactive apoprotein than holocytochrome. Similar findings have been reported by others working with liver cell cultures [12]. The different response of cultured hepatocytes to PB and metyrapone is most evident after 72 h, when the level of the *P*450IIB subfamily was undetectable and the hepatocytes' response to PB and metyrapone was, like that to BNF, to increase the relative proportion of the *P*450IA subfamily (cf. Tables 1 and 2). These observations are compatible with the general consensus [13,14] that cultured hepatocytes are refractory to PB type inducers. However, the current work does show that metyrapone behaves like a PB-type inducer in rat liver and that, unlike the classical inducers, it can prevent the loss of the cytochrome *P*-450 content in hepatocyte culture.

The finding that metyrapone elevates the level of the *P*450IA subfamily after 72 h of culture is compatible with the pronounced induction of ethoxyresorufin *O*-de-ethylase activity observed previously [2,4]. However, the ability of metyrapone to maintain the activity of ethylmorphine *N*-demethylase in hepatocyte culture is not commensurate with its effects on the *P*450IIB subfamily. This subfamily has generally been considered to be associated with ethylmorphine *N*-demethylase. However, it is interesting to note that the *P*450IIIA subfamily has also been shown to have a pronounced ethylmorphine *N*-demethylase activity [15].

In conclusion, the present work shows that the effects of metyrapone on the relative proportions of the *P*450IA and *P*450IIB subfamilies in hepatocyte culture are not completely in accord with its effects on the total amount of the cytochrome. Thus further work is required to characterize the effects of metyrapone on the other subfamilies of hepatic cytochromes *P*-450, particularly the IIIA isoenzyme, in order to understand the mechanisms underlying the loss of the cytochrome in hepatocyte culture and the ability of metyrapone to prevent this from occurring.

REFERENCES

1. Netter, K. J. (1980) *Pharmacol. Ther.* **10**, 515–536
2. Lake, B. G. & Paine, A. J. (1983) *Xenobiotica* **13**, 725–730
3. Paine, A. J., Villa, P. & Hockin, L. J. (1980) *Biochem. J.* **188**, 937–939
4. Lake, B. G. & Paine, A. J. (1982) *Biochem. Pharmacol.* **31**, 2141–2144

5. Guengerich, F. P., Dannan, G. A., Wright, S. T., Martin, M. V. & Kaminsky, L. S. (1982) *Biochemistry* **21**, 6019–6030
6. Stephens, G. T. (1989) M. Phil. Thesis, University of London
7. Paine, A. J., Allen, C. M., Durkacz, B. W. & Shall, S. (1982) *Biochem. J.* **202**, 551–553
8. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350–4354
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
10. Black, S. D. & Coon, M. J. (1986) in *Cytochrome P450* (Ortiz de Montellano, P. R., ed.), pp. 161–216, Plenum Press, New York
11. Pickett, C. B., Jeter, R. L., Morin, J. & Lu, A. Y. H. (1981) *J. Biol. Chem.* **256**, 8815–8820
12. Steward, A. R., Dannan, G. A., Guzelian, P. S. & Guengerich, F. P. (1985) *Mol. Pharmacol.* **27**, 125–132
13. Nebert, D. W. & Gonzalez, F. J. (1987) *Annu. Rev. Biochem.* **56**, 945–993
14. Turner, N. A., Wilson, N. M., Jefcoate, C. R. & Pitot, H. C. (1988) *Arch. Biochem. Biophys.* **263**, 204–215
15. Wrightson, S. A., Schuetz, E. G., Watkins, P. B., Maurel, P., Barwick, J., Bailey, B. S., Hartle, H. T., Young, B. & Guzelian, P. S. (1985) *Mol. Pharmacol.* **28**, 312–321

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