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BINDING OF METYRAPONE TO DITHIONITE-REDUCED CYTOCHROME P-450 FROM RATS TREATED WITH XENOBIOTICS

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Abstract—The *in vitro* binding of metyrapone to dithionite-reduced cytochrome P-450 in hepatic microsomes from rats treated *in vivo* with thirteen different xenobiotics was studied spectrophotometrically. The proportion of cytochrome P-450 that bound metyrapone increased 1.8-fold to about 78% following treatment with phenobarbitone (PB) and PB-type inducers (*trans*-stilbene oxide, 2,2',4,4'-tetrachloro-, 2,2',4,5,5'-pentabromo- and 2,2',4,4',5,5'-hexachlorobiphenyl) but remained unaltered following treatment with 3-methylcholanthrene (MC) and MC-type inducers (benzo[*a*]pyrene, β-naphthoflavone and 3,3',4,4'-tetrabromobiphenyl). The simultaneous induction of the PB-inducible and MC-inducible forms of cytochrome P-450 by administering Aroclor 1254 or by coadministering PB with MC increased the proportion of cytochrome P-450 that bound metyrapone to 74 and 78% respectively. PB treatment increased whereas MC treatment decrease the binding affinity for metyrapone by approximately 20-fold. Treatment with isosafrole or metyrapone itself failed to stimulate metyrapone binding. In contrast, pregnenolone-16α-carbonitrile was indistinguishable from PB in its ability to increase the binding capacity and binding affinity for metyrapone. Our results indicate that metyrapone binding is not specific for cytochrome P-450b, the major PB-inducible hemoprotein, as has been proposed [V. Luu-The, J. Cumps and P. Dumont, *Biochem. biophys. Res. Commun.* **93**, 776 (1980)].

Cytochrome P-450-dependent monooxygenases are inducible microsomal enzymes that catalyse the oxication of a multitude of structurally diverse substrates including chemical carcinogens [1, 2]. This broad substrate specificity reflects the existence of multiple forms of cytochrome P-450, and six forms from rat liver have been purified and characterised [3–8]. These six forms, five of which are arbitrarily designated cytochrome P-450a–P-450e, are inducible to varying degrees by different xenobiotics as summarised in Table 1.

Unfortunately, the individual forms of cytochrome P-450 present in crude microsomes are not readily distinguishable from each other because they display overlapping substrate specificities and similarities both in molecular weight and ligand-binding (spectral) characteristics. Monospecific antibodies permit the quantitation of individual forms of cytochrome P-450 by techniques such as radial immunodiffusion. Such immunochemical analysis was first reported by Thomas *et al.* [9, 10] for the quantitation of cytochromes P-450a, P-450b, and P-450c and is currently the only method of directly and unabiguously quantitating several individual forms of cytochrome P-450 in microsomes. Although immunochemical techiques provide the specificity necessary to distinguish individual isozymes of cytochrome P-450, such techniques, as they are currently employed, suffer the disadvantage of being relatively slow and unsuitable for screening a large number of samples.

Recently, Luu-The *et al.* [11, 12] reported that metyrapone forms a spectrally apparent complex (λ_{max} = 446 nm) with dithionite-reduced cytochrome P-450b (the major PB-inducible hemoprotein) but not with cytochrome P-450c (the major MC-inducible hemoprotein) or cytochrome P-450a (a minor PB- and MC-inducible hemoprotein).§ Based on this observation these authors described a simple and rapid method for the specific determination of cytochrome P-450b in hepatic microsomes. To evaluate the specificity of the interaction between metyrapone and reduced cytochrome P-450, we examined the metyrapone-difference spectrum of dithionite-reduced microsomes following the differential induction of cytochrome P-450b (with five PB-type inducers), cytochrome P-450c (with four MC-type inducers), cytochrome P-450d (with isosafrole) or PCN–cytochrome P-450 (with PCN).* The effects of treating rats with Aroclor 1254 or metyrapone itself on the binding of metyrapone to hepatic microsomal ferrocytochrome P-450 were also examined. The results demonstrate that metyrapone binds with high affinity to forms of cytochrome P-450 other than cytochrome P-450b.

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§ Abbreviations: B[*a*]P, benzo[*a*]pyrene; BNF, β-naphthoflavone; CO, carbon monoxide; HCBP, 2,2',4,4',5,5'-hexachlorobiphenyl; MC, 3-methylcholanthrene; PB, phenobarbitone; PBBP, 2,2',4,5,5'-pentabromobiphenyl; PCN, pregnenolone-16α-carbonitrile; TBBP, 3,3',4,4'-tetrabromobiphenyl; TCBP, 2,2',4,4'-tetrachlorobiphenyl; and TSO, *trans*-stilbene oxide.

Table 1. Inducibility of individual forms of rat liver microsomal cytochrome P-450 by various xenobiotics

Cytochrome P-450	Inducibility	Ref.
Cytochrome P-450a	Minor PB- and MC-inducible hemoprotein	9
Cytochrome P-450b	Major PB-inducible hemoprotein	9, 10
Cytochrome P-450c*	Major MC-inducible hemoprotein	9, 10
Cytochrome P-450d	Major isosafrole-inducible hemoprotein	5
Cytochrome P-450e	Minor PB-inducible hemoprotein	6, 7
PCN–cytochrome P-450	Major PCN-inducible hemoprotein	8

* Cytochrome P-450c is also known as cytochrome P-448 and cytochrome P_1-450.

MATERIALS AND METHODS

3-Methylcholanthrene (MC) and benzo[*a*]pyrene (B[*a*]P) were purchased from the Sigma Chemical Co. (St. Louis, MO); β-naphthoflavone (BNF), *trans*-silbene oxide (TSO) and metyrapone (2-methyl-1,2-di-[3-pyridyl]-1-propanone) from the Aldrich Chemical Co (Milwaukee, WI); carbon monoxide (CO) from Matheson (East Rutherford, NJ); and isosafrole from Eastman Organic Chemicals (Rochester, NY). Phenobarbitone (PB) was obtained from Elkins-Sinn Inc. (Cherry Hill, NJ). Pregnenolone-16α-carbonitrile (PCN) was a gift from G. D. Searle & Co. (Chicago, IL) and 2-*n*-heptylbenzimidazole was a gift from Dr. S. R. Challand of the Wellcome Research Laboratories (Beckenham, England). 2,2′,4,4′-Tetrachlorobiphenyl (TCBP) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (HCBP) were synthesised from 2,2′-dichloro- and 2,2′,4,4′-tetrachlorobenzidine, respectively, and were purified as previously described [14]. 3,3′,4,4′-Tetrabromobiphenyl (TBBP) was synthesised by the Cadogan coupling [15] of 3,4-dibromoaniline (Pfaltz & Bauer, Inc., Stamford, CT) with excess 1,2-dibromobenzene (Aldrich) and was purified essentially as described for 3,3′,4,4′-tetrachlorobiphenyl [16]. 2,2′,4,5,5′-Pentabromobiphenyl (PBBP) was similarly synthesised by the Cadogan coupling of 2,4,5-tribromoaniline with excess 1,4-dibromobenzene. The synthesis of 2,4,5-tribromoaniline has been described elsewhere [17].

Animal treatment and isolation of microsomes. One-month-old male Wistar rats ((Woodlyn Laboratories, Ltd., Guelph, Ontario, Canada) were housed in wire cages and allowed free access to Purina Certified Rat Chow (No. 5002) and water. TCBP, HCBP, PBBP (150 μmoles/kg), TBBP (30 μmoles/kg) and Aroclor 1254 (750 μmoles/kg) were administered to rats by intraperitoneal injection on days 1 and 3. TSO (400 mg/kg) was administered on days 1 through 5 inclusive. Isosafrole (150 mg/kg), PCN (50 mg/kg), PB, metyrapone (400 μmoles/kg), MC, B[*a*]P and BNF (100 μmoles/kg) were injected on days 3, 4 and 5. All chemicals were administered in corn oil except PB for which isotonic saline was the vehicle. Controls received a corresponding volume of corn oil (5 ml/kg) on days 1 and 3. Rats were starved on day 5 to lower liver glycogen levels and were killed by cervical dislocation on day 6.

Rat livers were perfused via the hepatic portal vein with ice-cold isotonic saline supplemented with EDTA (0.1 mM). The blanched livers were homogenised in 0.25 M sucrose, 0.1 mM EDTA, pH 7.6. The microsomal fraction was collected as a 100,000 *g* pellet by further centrifugation of a 10,000 *g* supernatant fraction from the liver homogenate essentially as described [18]. Microsomes were resuspended in 0.25 M sucrose, 0.1 mM EDTA. Protein concentrations were determined by the method of Lowry *et al.* [19] with bovine serum albumin as standard. The cytochrome P-450 content was determined by the method of Omura and Sato [20] from the CO-difference spectrum of dithionite-reduced microsomes based on a millimolar extinction coefficient of 91 $mM^{-1} \cdot cm^{-1}$.

Metyrapone binding. The metyrapone-difference spectrum of dithionite-reduced microsomes was recorded at room temperature on a Cary 118C spectrophotometer equipped with a repetitive scan accessory. Microsomes were diluted to 3 or 6 mg protein/ml of 0.25 M sucrose, 0.1 mM EDTA. A 2-ml aliquot was further diluted with 4.8 ml potassium phosphate buffer (0.1 M, pH 7.4), reduced with a few grains of solid sodium dithionite and divided equally between two matched quartz cuvettes. After a baseline of equal light absorbance was established, metyrapone, dissolved in phosphate buffer, was added to the sample cuvette (final concentration 1 mM) while a corresponding volume (100 μl) of phosphate buffer was added to the reference cuvette. After a 2–5 min incubation period, the metyrapone-difference spectrum was recorded between 400 and 500 nm. From the increase in absorbance at 446 nm (A_{446}–A_{490}), the concentration of metyrapone complex was determined based on a millimolar extinction coefficient of 52 $mM^{-1} \cdot cm^{-1}$ [12].

To determine K_s (the concentration of metyrapone causing half-maximal spectral interaction with reduced microsomes), various concentrations of metyrapone (10^{-7} to 10^{-4} M) were added cumulatively in 1-μl aliquots (using a Hamilton microsyr-

* The nomenclature of Ryan *et al.* is used in Table 1. However, Vlasuk *et al.* [13] demonstrated recently that a major PB-inducible form of cytochrome P-450 in Long Evans rats differs slightly in structure and molecular weight from that in Holtzman rats. In view of this strain difference and the fact that Wistar rats were used in the present study, we do not wish to imply that, in using the nomenclature of Ryan *et al.*, the various forms of cytochrome P-450 in the Long Evans rat and the responsiveness of this strain to various xenobiotics are necessarily identical in the Wistar rat.

Table 2. Effect of phenobarbitone (PB) and PB-type inducers on the binding of metyrapone to dithionite-reduced microsomes*

Treatment	Concentration of metyrapone complex† (μM)	Concentration of CO complex‡ (μM)	% cytochrome P-450 bound to metyrapone
Control			
Corn oil	0.28 ± 0.03	0.63 ± 0.01	44 ± 3
PB-type inducers			
PB	1.2 ± 0.1§	1.6 ± 0.1§	78 ± 6§
TCBP	1.2 ± 0.2§	1.6 ± 0.2§	78 ± 5§
PBBP	1.2 ± 0.2§	1.5 ± 0.1§	80 ± 4§
HCBP	1.3 ± 0.2§	1.7 ± 0.2§	76 ± 5§
TSO	0.85 ± 0.6§	1.1 ± 0.1	77 ± 3§

* Values are means ± S.D. of four to five determinations.

† Calculated from the increase in absorbance at 446 nm (A_{446}–A_{490}) using the millimolar extinction coefficient 52 $mM^{-1} \cdot cm^{-1}$ [12].

‡ Calculated from the increase in absorbance at 450 nm (A_{450}–A_{490}) using the millimolar extinction coefficient 91 $mM^{-1} \cdot cm^{-1}$ [20].

§ Significantly different ($P < 0.05$) from values for corn oil treated rats.

inge) to dithionite-reduced microsomes (pooled from three animals at a final concentration of 1 mg protein/ml). Apparent K_s values were determined from double-reciprocal plots of the increase in absorbance at 446 nm (A_{446}–A_{490}) versus metyrapone concentration.

Displacement of the isosafrole metabolite from cytochrome P-450. To assess more accurately the ligand-binding properties of microsomes isolated from rats pretreated with isosafrole, the isosafrole metabolite formed *in vivo* [21] was displaced from cytochrome P-450 *in vitro* with 2-*n*-heptylbenzimidazole, the most effective displacer known [22]. 2-*n*-Heptylbenzimidazole, dissolved in *N*-*N*-dimethylformamide, was added to 10 ml of microsomes (1 mg protein/ml phosphate buffer, pH 7.4) which had been preincubated at 37° for 5 min as described [22]. The incubation with 2-*n*-heptylbenzimidazole (final concentration 200 μM) was continued for a further 20 min after which the CO- or metyrapone-difference spectrum was recorded as described above.

Statistical analysis. The statistical significance between the sample means of control and treated groups for each variable studied was analysed by Student's *t*-test at the 5% level of significance ($P < 0.05$).

RESULTS

The addition of metyrapone to dithionite-reduced microsomes from rat liver resulted in the formation of a stable ferrocytochrome P-450–metyrapone complex which absorbed maximally at 446 nm. As previously reported [23], the development of the 446 nm peak was preceded by formation of a peak at approximately 425 nm. The rate of conversion of the 425 nm to the 446 nm peak declined following treatment of rats with MC-type inducers of cytochrome P-450c. The period of incubation of metyrapone with dithionite-reduced microsomes was varied from 2 to 5 min to accommodate such differences in the rate of development of the 446 nm peak.

Treatment of rats with PB, TCBP, PBBP or HCBP increased the concentration of cytochrome P-450 approximately 2.5-fold (Table 2). The increase in cytochrome P-450 (λ_{max} = 450 nm) was accompanied

Table 3. Effect of 3-methylcholanthrene (MC) and MC-type inducers on the binding of metyrapone to dithionite-reduced microsomes*

Treatment	Concentration of metyrapone complex† (μM)	Concentration of CO complex‡ (μM)	% cytochrome P-450 bound to metyrapone
Control			
Corn oil	0.28 ± 0.03	0.63 ± 0.01	44 ± 3
MC-type inducers			
MC	0.62 ± 0.04§	1.3 ± 0.2§	48 ± 7
B[*a*]P	0.53 ± 0.04§	1.1 ± 0.1§	48 ± 4
BNF	0.50 ± 0.03§	1.1 ± 0.2§	45 ± 2
TBBP	0.73 ± 0.06§	1.5 ± 0.1§	49 ± 6

* Values are means ± S.D. of four to five determinations.

† Calculated from the increase in absorbance at 446 nm (A_{446}–A_{490}) using the millimolar extinction coefficient 52 $mM^{-1} \cdot cm^{-1}$ [12].

‡ Calculated from the increase in absorbance at 448–450 nm (A_{max}–A_{490}) using the millimolar extinction coefficient 91 $mM^{-1} \cdot cm^{-1}$ [20].

§ Significantly different ($P < 0.05$) from values for corn oil treated rats.

Table 4. Effects of mixed-type inducers and other xenobiotics on the binding of metyrapone to dithionite-reduced microsomes*

Treatment	Concentration of metyrapone complex† (μM)	Concentration of CO complex‡ (μM)	% cytochrome P-450 bound to metyrapone
Control			
Corn oil	0.28 ± 0.03	0.63 ± 0.01	44 ± 3
Mixed-type inducers			
Aroclor 1254	1.7 ± 0.1§	2.3 ± 0.3§	74 ± 4§
PB + MC	1.8 ± 0.1§	2.3 ± 0.2§	78 ± 5§
Others			
Metyrapone	0.25 ± 0.02	0.60 ± 0.02	42 ± 5
PCN	1.0 ± 0.1§	1.25 ± 0§	80 ± 7§
Isosafrole −displacer	0.46 ± 0.03§	0.83 ± 0.03§	55 ± 3§
Isosafrole +displacer	0.45 ± 0.06§	1.35 ± 0.15§	33 ± 4§

* Values are means ± S.D. of four to five determinations.

§ Calculated from the increase in absorbance at 446 nm (A_{446}–A_{490}) using the millimolar extinction coefficient 52 $mM^{-1} \cdot cm^{-1}$ [12].

‡ Calculated from the increase in absorbance at 450 nm (A_{max}–A_{490}) using the millimolar extinction coefficient 91 $mM^{-1} \cdot cm^{-1}$ [20].

§ Significantly different ($P < 0.05$) from values for corn oil treated rats.

by about a 4.3-fold increase in the concentration of metyrapone complex. Following treatment of rats with these PB-type inducers, the proportion of cytochrome P-450 that bound to metyrapone was increased 1.8-fold from 44% (control) to approximately 78%. Similar results were obtained with TSO although this chemical, which is a potent inducer of epoxide hydrolase [9, 24, 25], was a less potent inducer of cytochrome P-450 than PB.

In contrast to the PB-type inducers, the MC-type inducers tested, namely MC, B[*a*]P, BNF and TBBP, did not cause a statistically significant ($P < 0.05$) increase in the proportion of cytochrome P-450 that bound metyrapone (Table 3).

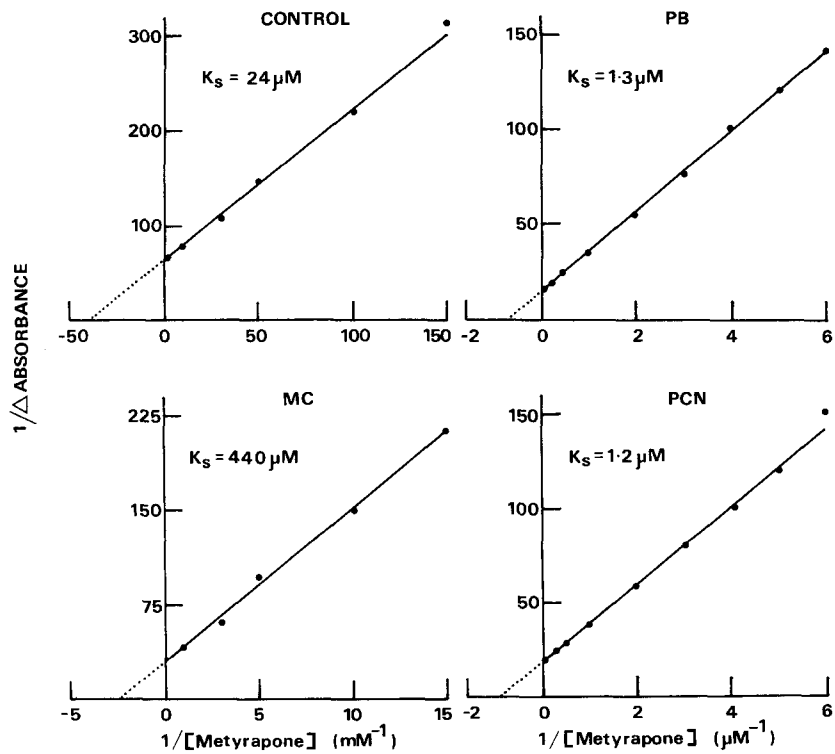

Fig. 1. Double-reciprocal plot of absorbance change (A_{446}–A_{490}) versus metyrapone concentration in control microsomes (top left) or microsomes from rats pretreated with phenobarbitone (PB, top right), 3-methylcholanthrene (MC, bottom left) or pregnenolone-16α-carbonitrile (PCN, bottom right). The abscissa units are mM^{-1} on the left (control and MC) and μM^{-1} on the right (PB and PCN).

The simultaneous induction of the PB-inducible and MC-inducible forms of cytochrome P-450 by administering Aroclor 1254 or by coadministering PB with MC increased the concentration of metyrapone complex and cytochrome P-450 by *ca.* 6.25- and 3.65-fold respectively. Following these treatments, the proportion of total cytochrome that bound metyrapone increased to 74–78% (Table 4).

The effects of treatment of rats with isosafrole, PCN or metyrapone on the binding of metyrapone to cytochrome P-450 are also shown in Table 4. As previously reported [21, 22], the extent of cytochrome P-450 induction by isosafrole was not fully realised until the isosafrole metabolite formed *in vivo* was displaced from cytochrome P-450 *in vitro* by 2-*n*-heptylbenzimidazole. Displacement of the isosafrole metabolite resulted in a 63% increase in CO-binding capacity, whereas the binding of metyrapone remained unchanged. Following displacement, the proportion of cytochrome P-450 that bound metyrapone in microsomes from isosafrole-induced rats was decreased to 33% (Table 4).

Like PB treatment, PCN treatment increased both cytochrome P-450 levels and metyrapone binding such that the proportion of cytochrome P-450 that bound metyrapone increased to 80% (Table 4).

Metyrapone, administered *in vivo*, neither induced cytochrome P-450 nor stimulated its binding to hepatic microsomes *in vitro* (Table 4). The rate of *N*-demethylation of aminopyrine (Aldrich) catalysed by microsomes from rats treated with metyrapone (4.84 ± 0.54 nmoles HCHO formed·(mg microsomal protein)$^{-1}$·min^{-1}) indicated no inhibition of this pathway when compared to control microsomes (4.44 ± 0.37).

Treatment of rats with PB or PCN influenced metyrapone binding in a similar manner (Tables 2 and 4), despite the fact that these xenobitics induce different forms of cytochrome P-450 [8, 9]. To compare further these two inducers, the metyrapone-binding affinity (K_s) of ferrocytochrome P-450 in microsomes from PB- and PCN-induced rats was measured (Fig. 1). The concentrations of metyrapone effecting half-maximal complex formation (K_s) were 24, 1.3 and 1.2 μM for microsomes from control, PB-induced and PCN-induced rats respectively.* These results indicate that both PB and PCN markedly increased the binding affinity of ferrocytochrome P-450 for metyrapone.

DISCUSSION

The *in vitro* binding of metyrapone to cytochrome P-450 using hepatic microsomes from rats treated *in vivo* with thirteen different xenobiotics was studied to test the proposal of Luu-The *et al.* [11, 12] that metyrapone binds specifically to cytochrome P-450b, the major PB-inducible hemoprotein. Following the induction of cytochrome P-450b by PB and PB-type inducers (TCBP, PBBP, HCBP and TSO), the proportion of cytochrome P-450 that, when reduced, bound metyrapone increased from 44% (control) to about 78% (Table 2). In contrast, no statistically significant ($P < 0.05$) increase in the proportion of cytochrome P-450 that bound metyrapone was observed following the induction of cytochrome P-450c by MC and MC-type inducers (B[*a*]P, BNF and TBBP) (Table 3). Measurement of the apparent binding affinity of dithionite-reduced cytochrome P-450 for metyrapone, which increased *ca.* 20-fold following PB treatment but decreased *ca.* 20-fold following MC treatment, also served to differentiate between the induction of cytochrome P-450b and cytochrome P-450c (Fig. 1). These results are consistent with those previously reported, with one exception [12, 23, 27]. In contrast to the linear double-reciprocal plots shown in Fig. 1, Estabrook and coworkers [27] obtained curvilinear Hofstee plots (A_{446}–A_{500} vs A_{446}–A_{500}/[metyrapone]) for hepatic microsomes from PB- or MC-treated rats. Since essentially linear plots were obtained when the data presented in Fig. 1 were analysed according to Hofstee and since a wide range of metyrapone concentrations was used, we are unable to explain the discrepancy between our results and those of Estabrook and coworkers [27]. Despite this discrepancy, the results of both studies demonstrate that microsomes from PB-treated rats have a considerably higher binding affinity for metyrapone than do microsomes from MC-treated rats.

Treatment of rats with isosafrole, an inducer of cytochrome P-450d [5], caused a slight decrease in the proportion of cytochrome P-450 that bound metyrapone (Table 4). When the isosafrole metabolite formed *in vivo* was displaced from cytochrome P-450 *in vitro* with 2-*n*-heptylbenzimidazole, CO binding, but not metyrapone binding, was increased 63%. Since the isosafrole metabolite formed *in vivo* apparently binds to cytochrome P-450d preferentially over cytochromes P-450a, P-450b and P-450c [5], the opposing effects of 2-*n*-heptylbenzimidazole on CO and metyrapone binding suggest that cytochrome P-450d does not bind metyrapone. In support of this interpretation, Ryan *et al.* [7] reported recently that cytochrome P-450d, purified to electrophoretic homogeneity from isosafrole-treated rats, failed to form a ligand complex with metyrapone.

Like PB, PCN induced a form or forms of cytochrome P-450 with high affinity ($K_s \simeq 1$ μM) and increased binding capacity for metyrapone (Table 4 and Fig. 1). Elshourbagy and Guzelian [8] have isolated and purified a PCN-inducible form of cytochrome P-450 which can be distinguished by a variety of criteria from the major PB-inducible and MC-inducible hemoproteins. Furthermore, Thomas *et al.* [9] demonstrated that PCN does not induce cytochrome P-450b or P-450c. These observations indicate that the unique form(s) of PCN-inducible cytochrome P-450 is(are) indistinguishable from the

* The concentration of metyrapone effecting half-maximal complex formation may also be defined as S_{50}, which is related to K_s as follows:

$$K_s = S_{50} - 0.5\,[E]$$

where $[E]$ is the molar concentration of the enzyme [26]. For microsomes from control and MC-treated rats, K_s is not significantly different from S_{50}. However, for microsomes from PB-treated rats $K_s = 1.3 - 0.8 = 0.5$ μM and for microsomes from PCN-treated rats $K_s = 1.2 - 0.625 = 0.6$ μM.

PB-inducible hemoproteins based on formation of a ligand complex with metyrapone.

Recently, Ryan and coworkers* showed that cytochrome P-450e, a minor PB-inducible form of cytochrome P-450, also binds metyrapone with high affinity. These results indicate that at least three forms of cytochrome P-450, two inducible by PB (P-450b and P-450e) and one inducible by PCN, bind metyrapone with high affinity, We conclude, therefore, that, despite a marked differential interaction between metyrapone and various isozymes of cytochrome P-450, metyrapone binding is not specific for a single form of cytochrome P-450. The binding of metyrapone to several forms of cytochrome P-450 most likely explains why 40–50% of the cytochrome P-450 in microsomes from control rats can bind metyrapone despite the fact that cytochrome P-450b comprises less than 5% of these hemoproteins [9].

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REFERENCES

1. A. Y. H. Lu, R. Kuntzman and A. H. Conney, *Front. gastrointest. Res.* **2**, 1 (1976).
2. M. J. Coon, *Nutr. Rev.* **36**, 319 (1978).
3. D. E. Ryan, P. E. Thomas, D. Korzeniowski and W. Levin, *J. biol. Chem.* **254**, 1365 (1979).
4. L. H. Botelho, D. E. Ryan and W. Levin, *J. biol. Chem.* **254**, 5635 (1979).
5. D. E. Ryan, P. E. Thomas and W. Levin, *J. biol. Chem.* **255**, 7941 (1981).
6. D. E. Ryan and W. Levin, *Fedn Proc.* **40**, 1640 (1981).
7. D. E. Ryan, P. E. Thomas and W. Levin, *Archs Biochem. Biophys.*, in press.
8. N. A. Elshourbagy and P. S. Guzelian, *J. biol. Chem.* **255**, 1279 (1980).
9. P. E. Thomas, L. Reik, D. E. Ryan and W. Levin, *J. biol. Chem.* **256**, 1044 (1981).
10. P. E. Thomas, D. Korzeniowski, D. Ryan and W. Levin, *Archs Biochem. Biophys.* **192**, 524 (1979).
11. V. Luu-The, J. Cumps and P. Dumont, in *Microsomes, Drug Oxidations, and Chemical Carcinogenesis* (Eds. M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette and P. J. O'Brien), p. 1001. Academic Press, New York (1980).
12. V. Luu-The, J. Cumps and P. Dumont, *Biochem. biophys. Res. Commun.* **93**, 776 (1980).
13. A. P. Vlasuk, J. Ghrayeb, D. E. Ryan, L. Reik, P. E. Thomas, W. Levin and F. G. Walz, *Biochemistry* **21**, 789 (1982).
14. O. Hutzinger and S. Safe, *Bull. environ. Contam. Toxic.* **7**, 374 (1972).
15. J. I. G. Cadogan, *J. chem. Soc.* 4257 (1962).
16. A. Parkinson, R. Cockerline and S. Safe, *Chem. Biol. Interact.* **29**, 277 (1980).
17. L. W. Robertson, A. Parkinson and S. Safe, *Biochem. biophys. Res. Commun.* **92**, 175 (1980).
18. A. Parkinson and S. Safe, *J. Pharm. Pharmac.* **31**, 444 (1979).
19. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
20. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
21. C. R. Elcombe, J. W. Bridges, T. J. B. Gray, R. H. Nimmo-Smith and K. J. Netter, *Biochem. Pharmac.* **24**, 1427 (1975).
22. M. Dickens, C. R. Elcombe, S. J. Moloney, K. J. Netter and J. W. Bridges, *Biochem. Pharmac.* **28**, 231 (1979).
23. H. G. Jonen, B. Hüthwohl, R. Kahl and G. F. Kahl, *Biochem. Pharmac.* **23**, 1319 (1974).
24. H. Schmassmann and F. Oesch, *Molec. Pharmac.* **14**, 834 (1978).
25. H. Mukhtar, T. H. Elmamlouk and J. R. Bend, *Chem. Biol. Interact.* **22**, 125 (1978).
26. O. H. Straus and A. Goldstein, *J. gen Physiol.* **26**, 559 (1943).
27. J. Werringloer and R. W. Estabrook, in *The Induction of Drug Metabolism* (Eds. R. W. Estabrook and E. Lindenlaub), p. 269. F. K. Schattauer, Stuttgart, West Germany (1979).

* D. E. Ryan and W. Levin, personal communication.

Note added in proof—A recent paper [(K. M. Ivanetich, A. K. Costa and T. Brittain, *Biochem. biophys. Res. Commun.* **105**, 1322 (1982)] supports our conclusions.