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EFFECTS OF CYTOCHROME P-450 MONOOXYGENASE INDUCERS ON MOUSE HEPATIC MICROSOMAL METABOLISM OF TESTOSTERONE AND ALKOXYRESORUFINS

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Abstract—The effects of treatment with phenobarbital, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP), pregnenolone-16α-carbonitrile (PCN), 3-methylcholanthrene (3-MC) and isosafrole on the hepatic microsomal formation of nine monohydroxy metabolites of testosterone and the O-dealkylation of the ethyl and pentyl ethers of resourfin were evaluated in adult male C57BL/6J and DBA/2NCR mice. In both strains, phenobarbital, TCPOBOP and PCN induced testosterone 2β-, 6β-, 15β- and 16β-hydroxylases up to 5-fold, while phenobarbital and TCPOBOP increased the rate of dealkylation of pentoxyresorufin by approximately 30-fold. However, phenobarbital and TCPOBOP did not exhibit identical patterns of induction for the testosterone oxidation reactions. Hepatic microsomes from C57BL/6J mice treated with TCPOBOP displayed a depression in 6α-testosterone hydroxylase activity, which was also observed in PCN-treated animals, whereas phenobarbital-treated mice exhibited an elevation in this monooxygenase activity. A dose of TCPOBOP (0.5 μmol/kg) previously demonstrated to represent an ED_{50} for mouse aminopyrine *N*-demethylase activity was also found to approximate the ED_{50} for pentoxyresorufin *O*-dealkylase activity in the C57BL/6J mouse. Isosafrole or 3-MC treatment had little effect on testosterone metabolism or pentoxyresorufin *O*-dealkylase activity in either strain, while 3-MC induced ethoxyresorufin *O*-deethylase activity in C57BL/6J but not DBA/2NCR mice. This study confirms that TCPOBOP is a potent cytochrome P-450 inducer which most closely resembles phenobarbital in its mode of action. However, TCPOBOP and phenobarbital do not evoke identical modulations of cytochrome P-450-dependent monooxygenases in mice.

The cytochrome P-450 isozyme composition of the hepatic endoplasmic reticulum is affected markedly by exposure of the organism to xenobiotics. Treatment of rats with 3-methylcholanthrene (3-MC)† results in a marked increase in the microsomal content of cytochromes P-450a, P-450c and P-450d, whereas administration of phenobarbital elevates the concentration of cytochromes P-450a, P-450b, P-450e, P-450k and P-450p [1–6]. Pregnenolone-16α-carbonitrile (PCN) represents the prototype compound for a third class of cytochrome P-450 isozyme inducers, a class which primarily increases the level of cytochrome P-450p [4–6]. Although the mechanism of induction for the cytochrome P-450c gene has been determined to involve the interaction of a ligand–receptor complex with genomic regulatory elements, the process by which phenobarbital and PCN exert their inductive effect is unknown [7–12].

Recently, the compound 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) has been demonstrated in mice to be much more potent than phenobarbital as an inducer of several cytochrome P-450-mediated activities [13–16]. This has led to the suggestion that the induction of these activities may proceed through a receptor mechanism. TCPOBOP appears to duplicate numerous hepatic effects of phenobarbital such as the induction of phase II drug-metabolizing enzymes, increase in liver weight, proliferation of the smooth endoplasmic reticulum, and promotion of hepatocarcinogenesis [13, 17, 18]. Curiously, TCPOBOP appears much less active in producing this pleiotropic response in the rat and guinea pig than in the mouse and hamster [14].

Since the activities utilized to demonstrate enzyme induction by TCPOBOP, namely aminopyrine *N*-demethylase, aldrin epoxidase and benzphetamine *N*-demethylase, are catalyzed by more than one cytochrome P-450 isozyme in rats, it is possible that the pattern of isozyme induction by TCPOBOP is not identical to that produced by phenobarbital [19–21]. Isozyme specific activities have not been defined clearly in mice and, therefore, the present investigation has employed monooxygenase reactions identified as markers of rat cytochrome P-450 isozymes to examine in more detail the induction of murine cytochrome P-450 by TCPOBOP and phenobarbital. The substrates chosen were testosterone and the ethyl and pentyl ethers of resorufin. In the rat, the O-deethylation of ethoxyresorufin has been considered indicative of cytochrome P-450c activity, whereas the O-dealkylation of pentoxyresorufin has been suggested as isozyme specific for cytochrome P-450b [21, 22]. In addition, the following hydroxy-

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† Abbreviations: 3-MC, 3-methylcholanthrene; PCN, pregnenolone-16α-carbonitrile; TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene; B6, C57BL/6J; D2, DBA/2NCR; EROD, ethoxyresorufin *O*-deethylase; and PROD, pentoxyresorufin *O*-dealkylase.

lation reactions of testosterone have been suggested as isozyme specific for the rat proteins: 7α-hydroxylase, P-450a; 16β-hydroxylase, P-450b; 2α-hydroxylase, P-450h; and 6β-hydroxylase, P-450p [5, 23–25]. Although the orthologous proteins in the mouse may not exhibit identical catalytic specificity, an evaluation of these variables in response to phenobarbital, TCPOBOP, and PCN would refine the comparison of TCPOBOP and phenobarbital as monooxygenase inducers.

MATERIALS AND METHODS

Chemicals. TCPOBOP was synthesized according to a previously published procedure and was >97% pure as determined by gas chromatographic analysis [15]. PCN was furnished by the Upjohn Co. (Kalamazoo, MI). Isosafrole and resorufin were purchased from the Eastman Kodak Co. (Rochester, NY). Dexamethasone, 3-MC, testosterone, 16α-hydroxytestosterone and androstenedione were purchased from the Sigma Chemical Co. (St. Louis, MO). 2α-, 2β-, 6α- and 15α-Hydroxytestosterone were a gift of the Steroid Reference Collection, MRC (London, United Kingdom); 15β-hydroxytestosterone was a gift of Dr. Theo A. van der Hoeven (Albany Medical College, Albany, NY). 6β-, 7α- and 16β-Hydroxytestosterone were obtained from Steraloids, Inc. (Wilton, NH). Sodium phenobarbital was obtained from Elkins-Sinn, Inc. (Cherry Hill, NJ). The ethyl and pentyl ethers of resorufin were synthesized from resorufin and the appropriate alkyl iodides as described [26].

Animal treatment and isolation of microsomes. C57BL/6J (B6) mice, 8 weeks of age, were purchased from the Jackson Laboratory (Bar Harbor, ME). DBA/2NCR (D2) mice, 8 weeks of age, were obtained from Harlan Sprague–Dawley (Houston, TX). The mice were housed in plastic cages with hardwood bedding and allowed free access to Purina Rodent Chow, No. 5001, and water. They were maintained on a diurnal cycle of 12 hr of light and 12 hr of darkness.

All compounds were administered intraperitoneally. PCN, 3-MC and isosafrole, each dissolved in corn oil, were administered consecutively on days 1, 2, 3 and 4. Sodium phenobarbital was administered in saline solution on days 2, 3 and 4. TCPOBOP and 1,4-bis[2-(3-chloro,5-trifluoromethylpyridoyloxy)]benzene were dissolved in corn oil and administered as a single dose on day 1. The dose of each inducer is given in Tables 1–3. Administration of corn oil (20 mL/kg) on days 1, 2, 3 and 4 served as a control. Animals were killed by cervical dislocation on day 5. The livers were perfused *in situ* via the hepatic portal vein with 15 mL of ice-cold isotonic saline containing 0.1 mM EDTA. Livers were weighed, homogenized with a Potter–Elvehjem homogenizer in 4 vol. of 0.25 M sucrose containing 0.1 mM EDTA, and centrifuged at 10,000 *g* for 20 min. The 10,000 *g* supernatant fraction was centrifuged for 1 hr at 100,000 *g* and the microsomal pellet resuspended in the homogenizing solution. Aliquots of the microsomal suspension were stored at −70° until catalytic properties were measured.

Assays. Ethoxyresorufin *O*-deethylase activity was measured by the method of Pohl and Fouts [27]. The assay for pentoxyresorufin *O*-dealkylase was carried out in a manner identical to the ethoxyresorufin *O*-deethylase assay except that pentoxyresorufin (8 μM) replaced ethoxyresorufin in the incubation mixture.

Testosterone hydroxylase activities were determined as the rate of formation of the corresponding oxidation product [23, 28]. Product formation for all testosterone hydroxylase activities was linear with respect to time of incubation and protein concentration. Incubation mixtures consisted of 25 mM potassium phosphate (pH 7.4), 100 mM sucrose, 1.5 mM magnesium chloride, 1 mM NADPH, 1 mM NADH, 30 μM EDTA, 250 μM testosterone and 0.9 mg microsomal protein in a final volume of 1.0 mL. The reaction was initiated by the addition of substrate in 0.04 mL methanol. Following an incubation period of 5 min at 37°, the reaction was stopped by the addition of 6 mL of dichloromethane which contained 500 nM dexamethasone as an internal standard. Tubes were capped and shaken on a rotating platform for 10 min. Following centrifugation (5 min, 800 *g*), 4 mL of the organic phase was transferred to a culture tube (16 × 100 mm) and evaporated to dryness under a stream of nitrogen. The residue was dissolved in 0.5 mL of methanol and 0.02 mL injected onto the high pressure liquid chromatography column.

All chromatographic analyses were performed with a Rabbit HP solvent delivery system (Rainin Instruments Co., Woburn, MA) equipped with two Rainin Rabbit HP pumps (5 mL pump head, analytical) and a Rainin Microcomputer Gradient Controller. Separation of testosterone metabolites was performed by modifications of the method devised by van der Hoeven [28], using a 5 μm octyldecylsilane reverse phase column (250 × 4.6 mm inner diameter, Regis Chemical Co., Morton Grove, IL) preceded by a guard column (50 × 3.2 mm) of 30–40 μm octyldecylsilane (Rainin Instruments Co.). Analysis was performed by gradient elution where solvent A contained 4.8% tetrahydrofuran (aqueous) and solvent B contained 4.8% tetrahydrofuran in methanol. The gradient was isocratic (40% B) from time 0–5 min and this was followed by a linear gradient (5–25 min) to 75% B. The column was maintained at this solvent ratio for 3 min and then passed through a linear gradient of 75–40% B over a period of 3 min. The column was allowed to reequilibrate at 40% B for 9 min prior to the next injection. The flow rate was kept constant at 0.8 mL/min. Separations were performed at room temperature (22–24°) and the column effluents were monitored at 254 nm employing a model 160 Absorbance Detector from Beckman Instruments (Palo Alto, CA). Recordings were made on a plotting/recording integrator (model 3390A, Hewlett Packard, Avondale, PA) set to the integration mode. Peak area ratios were calculated by dividing peak area of metabolites by peak area of the internal standard (dexamethasone). Metabolite formation was quantitated by interpolation from standard curves developed from peak area ratios of monohydroxytestosterone standards. The minimum quantifiable limit for the assay was defined as the lowest standard concentration for which a coefficient

of variation of less than 10% could be achieved. The minimum quantifiable limit for all monohydroxytestosterone standards was equivalent to an enzyme activity value of 30 pmol/mg protein/min and had a signal-to-noise ratio of greater than 10.

Protein concentrations were determined by the method of Lowry *et al.* [29] using bovine serum albumin as a standard.

Statistics. Dunnett's method [30] for multiple comparisons with a control was utilized to test for statistical significance.

RESULTS

The hepatic microsomal metabolism of testosterone in mature male and female B6 mice is outlined in Table 1. The rate of formation of androstenedione and the rate of hydroxylation at nine positions of the steroid nucleus were nearly equivalent between the sexes. However, the rate of 16β-hydroxylation was approximately 2-fold higher in male compared to female B6 mice. Also illustrated in Table 1 is the effect of treatment of female B6 mice with PCN and the effect of treatment of male B6 mice with PCN, isosafrole, 3-MC, TCPOBOP and phenobarbital. Treatment of female B6 mice with PCN resulted in a significant increase in the rate of formation of 2α-, 2β-, 6β-, 15β- and 16β-hydroxytestosterone and a significant decrease in the rate of production of 6α- and 7α-hydroxytestosterone. Male B6 treated with PCN demonstrated a significant increase in the rate of formation of 2β-, 6β-, 15β- and 16β-hydroxytestosterone and suppressed production of 6α-, 7α- and 15α-hydroxytestosterone.

Following treatment of B6 mice with isosafrole, the formation of 15α-hydroxytestosterone was suppressed slightly (~25%), whereas the oxidation of testosterone at the 17 position (i.e. androstenedione formation) was elevated by more than 2-fold. Administration of 3-MC to B6 mice resulted in nearly a 500% increase in the formation of adrostenedione. Treatment of B6 mice with TCPOBOP resulted in alterations of hepatic microsomal testosterone metabolism which qualitatively resembled the metabolite profile produced by PCN. The fold increase in metabolism at the 2β, 6β, 15β and 16β positions following TCPOBOP administration was approximately one-third less than the increase observed following PCN treatment. Similarly, the magnitude of the decrease in formation of 6α-hydroxytestosterone was less in TCPOBOP-treated mice compared to PCN-treated mice. The absence of suppression in the 15α-hydroxylation reaction represents the only qualitative discrepancy in testosterone metabolite profile between hepatic microsomes isolated from B6 mice treated with TCPOBOP or PCN. Treatment of B6 mice with phenobarbital elicited a significant increase in the rate of formation of 2β-, 15β- and 16β-hydroxytestosterone, similar to the results with PCN and TCPOBOP. However, unlike the latter compounds, no significant increase in the activity of 6β-testosterone was noted following phenobarbital treatment. Moreover, phenobarbital-treated animals exhibited enhanced androgen hydroxylation at the 6α and 15α positions in contrast to the PCN and TCPOBOP treatment groups. A decrease in the rate of formation of 7α-hydroxytestosterone was noted in the phenobarbital treatment group, but this effect was less marked than that noted for B6 mice pretreated with PCN or TCPOBOP.

An identical experimental protocol was utilized to examine hepatic microsomal testosterone metabolism in the D2 mouse strain (Table 2). Compared to males, female D2 mice possessed a lower rate of hydroxylation at the 16β position; this sex difference had also been noted in the B6 strain. However, in contrast to the latter strain, female D2 mice also demonstrated lower 2β-, 6β-, 15β- and 16α-testosterone hydroxylase activities and a 3-fold lower rate of androstenedione formation. Treatment of female D2 mice with PCN resulted in alterations of the testosterone metabolite profile which resembled the results for PCN-treated female B6 mice with three exceptions: no significant decrease in 7α-testosterone hydroxylase activity, greater than 200% elevation in 16α-testosterone hydroxylase activity and a marked increase in the rate of production of androstenedione. The effect of PCN administration on male D2 mice differed from similar treatment of male B6 mice in that male D2 mice exhibited an increased rate of 2α-hydroxylation and no significant depression in testosterone hydroxylase activity at the 6α, 7α or 15α positions.

Isosafrole treatment of D2 mice did not result in any significant modifications in the stereo- or regioselective metabolism of testosterone, whereas 3-MC treatment of D2 mice resulted in a statistically significant ($P < 0.05$) increase in the rate of oxidation of the steroid at the 17 position. Following administration of TCPOBOP or phenobarbital to D2 mice, the rate of androgen hydroxylation was increased at the 2α, 2β, 6β, 15β and 16β positions. In contrast to the results in the B6 mouse, the magnitude of the increase in metabolism at the 2β, 6β, 15β and 16β positions was greater following TCPOBOP or phenobarbital treatment compared to the increase observed following PCN treatment. Moreover, microsomes from TCPOBOP-treated D2 mice did not display a significant depression in the rate of formation of 6α- and 7α-hydroxytestosterone. Phenobarbital-pretreated D2 mice demonstrated elevated 6α- and 7α-testosterone hydroxylase activity; the former result paralleled that observed in the B6 mouse, whereas the latter result was in direct contrast to the depression of 7α-testosterone hydroxylase activity observed in the B6 mouse.

Microsomes from the treatment groups outlined above were also used to examine the O-dealkylation of ethoxy- and pentoxyresorufin. In both the B6 and D2 strains, treatment of female animals with PCN and male animals with TCPOBOP or phenobarbital resulted in a significant increase in pentoxyresorufin *O*-dealkylase activity (Tables 1 and 2). The inductive effect of PCN on female metabolism of pentoxyresorufin was small, approximately 3- to 6-fold, whereas TCPOBOP and phenobarbital elicited increases of 30- to 40-fold and 24- to 30-fold respectively. Administration of 3-MC to male B6 mice resulted in a 27-fold elevation of ethoxyresorufin *O*-deethylase activity, whereas PCN treatment of female mice elicited a small (~3-fold) but significant

Table 1. Hepatic microsomal metabolism of testosterone and alkoxyresorufins in the C57BL/6J mouse: Sex differences and response to inducers

Sex	Treatment	Testosterone (pmol/mg protein/min)										Alkoxyresorufin (pmol/mg protein/min)	
		2α*	2β	6α	6β	7α	15α	15β	16α	16β	Androstenedione	EROD	PROD
M	Corn oil 20 mL/kg	129† ± 20	465 ± 110	443 ± 57	3,505 ± 888	220 ± 23	229 ± 30	208 ± 45	180 ± 41	209 ± 56	1,341 ± 271	207 ± 29	35 ± 4
F	Corn oil 20 mL/kg	133 ± 15	407 ± 59	518 ± 228	2,789 ± 415	289 ± 30	213 ± 36	190 ± 26	154 ± 13	89 ± 20	1,606 ± 208	162 ± 35	48 ± 5
M	PCN 25 mg/kg/day	156 ± 6	1,305‡ ± 103	225‡ ± 20	10,543‡ ± 959	123‡ ± 12	154‡ ± 14	473‡ ± 38	192 ± 26	558‡ ± 67	1,015 ± 172	363 ± 87	97 ± 17
F	PCN 25 mg/kg/day	163‡ ± 10	1,458‡ ± 63	294‡ ± 51	11,710‡ ± 521	217‡ ± 30	194 ± 67	527‡ ± 23	201 ± 74	600‡ ± 37	2,462 ± 1,076	461‡ ± 26	202‡ ± 8
M	Isosafrole 150 mg/kg/day	117 ± 31	349 ± 164	437 ± 66	2,951 ± 1,339	199 ± 26	170§ ± 42	161 ± 51	297 ± 161	240 ± 83	3,159§ ± 2,014	417 ± 49	138 ± 28
M	3-MC 25 mg/kg/day	127 ± 19	286 ± 42	523 ± 63	2,893 ± 381	196 ± 21	237 ± 11	151 ± 19	291 ± 41	172 ± 27	6,466‡ ± 1,278	5,713‡ ± 548	160§ ± 5
M	TCPOBOP 20 μmol/kg	147 ± 30	911‡ ± 79	332§ ± 27	7,235‡ ± 727	111‡ ± 13	243 ± 48	356‡ ± 22	229 ± 8	476‡ ± 59	2,057 ± 155	338 ± 43	1,466‡ ± 124
M	Phenobarbital 40 μmol/kg/day	113 ± 2	698§ ± 109	546§ ± 53	4,862 ± 398	171‡ ± 11	349‡ ± 31	286§ ± 20	222 ± 19	424‡ ± 13	1,751 ± 221	243 ± 25	1,025‡ ± 77

Animals were treated with the inducers listed above as described in Materials and Methods. Abbreviations: EROD, ethoxyresorufin *O*-deethylase; and PROD, pentoxyresorufin *O*-dealkylase.

* With the exception of androstenedione, the abbreviations for testosterone denote the hydroxylated metabolite formed.

† Values are means ± SD for 4–5 mice.

‡ Significantly different from control value, $P \leq 0.01$.

§ Significantly different from control value, $P \leq 0.05$.

Table 2. Hepatic microsomal metabolism of testosterone and alkoxyresorufins in the DBA/2NCR mouse: Sex differences and response to inducers

Sex	Treatment	Testosterone (pmol/mg protein/min)										Alkoxyresorufin (pmol/mg protein/min)	
		2α*	2β	6α	6β	7α	15α	15β	16α	16β	Androstenedione	EROD	PROD
M	Corn oil 20 mL/kg	50† ± 11	341 ± 25	193 ± 83	5,584 ± 680	225 ± 64	70 ± 33	141 ± 22	310 ± 91	291 ± 40	1,121 ± 326	317 ± 17	35 ± 4
F	Corn oil 20 mL/kg	35 ± 4	202 ± 50	198 ± 63	3,188 ± 839	205 ± 53	96 ± 24	87 ± 23	75 ± 32	142 ± 56	343 ± 169	239 ± 31	31 ± 3
M	PCN 25 mg/kg/day	82‡ ± 3	748‡ ± 58	76 ± 5	12,653‡ ± 655	195 ± 34	67 ± 17	303‡ ± 24	300 ± 116	605‡ ± 36	932 ± 503	214‡ ± 13	89 ± 8
F	PCN 25 mg/kg/day	91‡ ± 5	827‡ ± 49	101§ ± 28	14,132‡ ± 712	287 ± 52	103 ± 15	333‡ ± 16	264‡ ± 52	620‡ ± 65	1,304‡ ± 276	219 ± 29	94‡ ± 10
M	Isosafrole 150 mg/kg/day	54 ± 7	394 ± 52	200 ± 38	6,263 ± 816	237 ± 38	62 ± 18	156 ± 20	354 ± 111	323 ± 34	2,033 ± 361	101‡ ± 25	51 ± 7
M	3-MC 25 mg/kg/day	55 ± 14	255 ± 51	233 ± 46	4,376 ± 936	197 ± 42	76 ± 28	92 ± 21	438 ± 132	297 ± 59	2,293§ ± 811	167‡ ± 21	31 ± 3
M	TCPOBOP 20 μmol/kg	109‡ ± 5	1,069‡ ± 130	259 ± 64	18,805‡ ± 2,155	263 ± 26	85 ± 17	467‡ ± 59	497§ ± 36	755‡ ± 54	1,676 ± 272	404‡ ± 13	1,078‡ ± 117
M	Phenobarbital 40 μmol/kg/day	109‡ ± 12	1,015‡ ± 156	450‡ ± 156	17,077‡ ± 2,630	318§ ± 49	113 ± 37	441‡ ± 74	490 ± 78	881‡ ± 157	992 ± 625	469‡ ± 79	839‡ ± 48

Animals were treated with the inducers listed above as described in Materials and Methods. Abbreviations: EROD, ethoxyresorufin *O*-deethylase; and PROD, pentoxyresourfin *O*-dealkylase.

* With the exception of androstenedione, the abbreviations for testosterone denote the hydroxylated metabolite formed.

† Values are means ± SD for 4–5 mice.

‡ Significantly different from control value, $P \leq 0.01$.

§ Significantly different from control value, $P \leq 0.05$.

Table 3. Effect of TCPOBOP and 1,4-bis[2-(3-chloro,5-trifluoromethylpyridyloxy)]benzene on hepatic microsomal pentoxyresorufin *O*-dealkylase activity in male C57BL/6J mice

Treatment	Dose	Pentoxyresorufin *O*-dealkylase (pmol/mg protein/min)
Corn oil	20 mL/kg	28 ± 2*
TCPOBOP	0.5 μmol/kg	782 ± 55†
TCPOBOP	2.5 μmol/kg	1315 ± 104†
1,4-Bis[2-(3-chloro,5-trifluoromethylpyridyloxy)]benzene	0.5 μmol/kg	45 ± 7
1,4-Bis[2-(3-chloro,5-trifluoromethylpyridyloxy)]benzene	2.5 μmol/kg	362 ± 44†

Animals were treated with the inducers listed above as described in Materials and Methods.
* Values are means ± SD for 4 mice.
† Significantly different from control value, $P \leq 0.01$.

increase in this enzyme activity (Table 1). Treatment of male B6 mice with PCN, isosafrole, TCPOBOP or phenobarbital did not evoke a significant increase in *O*-deethylase activity. A depression in this enzyme activity was noted in male D2 mice following treatment with PCN, isosafrole and 3-MC (Table 2). However, D2 mice demonstrated a 30–50% increase in ethoxyresorufin O-dealkylation following administration of TCPOBOP or phenobarbital.

The O-dealkylation of pentoxyresorufin was also examined in B6 mice treated with lower doses of TCPOBOP (Table 3). A single dose of TCPOBOP (0.5 μmol/kg), equivalent to the estimated ED_{50} of this compound for aminopyrine *N*-demethylase activity, produced a 28-fold rise in the O-dealkylation reaction, whereas a single dose of 2.5 μmol/kg appeared to elicit an increase in this activity equivalent to that produced by 20 μmol/kg (Tables 1 and 3). A TCPOBOP analog with substitution of a trifluoromethyl group at the 5 position of the pyridine ring was also utilized as an inducer in this experiment. Similar to other TCPOBOP analogs, this compound was considerably less potent than the parent compound as a monooxygenase inducer [15, 16].

DISCUSSION

Ethoxyresorufin *O*-deethylase activity was highly induced by 3-MC in the B6 mouse but only slightly affected in the other treatment groups of this strain or any treatment group of the D2 strain. This result is consistent with the finding of Poland *et al.* [13] that TCPOBOP does not induce aryl hydrocarbon hydroxylase activity in the B6D2F1/J mouse and suggests that TCPOBOP does not interact with the Ah receptor. The large induction of pentoxyresorufin *O*-dealkylase activity by TCPOBOP and phenobarbital in both the B6 and D2 strains further demonstrates the similarity of these inducers. It is important to note that PCN produces only a slight increase in pentoxyresorufin *O*-dealkylase activity; other monooxygenase activities such as aminopyrine *N*-demethylase and aldrin epoxidase do not distinguish between induction by phenobarbital and PCN [19, 21].

An examination of the testosterone metabolism data in both strains for the five monooxygenase inducers indicates the following: (1) 2β-, 15β- and 16β-testosterone hydroxylase activities were induced efficiently by not only TCPOBOP and phenobarbital but also by PCN, (2) for one activity in the B6 strain, namely testosterone 6α-hydroxylase, TCPOBOP resembled PCN by producing a depression of this activity whereas phenobarbital induced this activity, and (3) 3-MC elicited an increase in the rate of 17-oxidation in the B6 strain and a more moderate increase in the D2 strain. Therefore, 2β-, 15β- and 16β-testosterone hydroxylase activities resemble other monooxygenase activities such as aldrin epoxidase and aminopyrine *N*-demethylase. None of these activities can be utilized to categorize an inducer as PCN-like versus phenobarbital-like. However, 6α-hydroxylation of testosterone was modulated differently by phenobarbital and PCN. For this reaction, TCPOBOP and PCN depressed this activity, whereas phenobarbital induced this activity. Finally, it is of interest to note that 3-MC increased the rate of formation of androstenedione in the mouse, whereas this effect is not observed in the rat [23].

The administration of 0.5 μmol/kg of TCPOBOP to the B6 mouse resulted in an increase in pentoxyresorufin *O*-dealkylase activity to approximately 50% of maximal activity (i.e. 0.5 μmol/kg approximates an ED_{50} dose for this activity). Therefore, TCPOBOP induces at least one monooxygenase activity which is unique to a "phenobarbital-like" response at a dosage far less than that required for phenobarbital. In addition, both compounds induce a second set of cytochrome P-450 mediated monooxygenase activities which are not unique to a "phenobarbital-like" response. For these activities as well (e.g. aminopyrine *N*-demethylase, aldrin epoxidase) TCPOBOP maintains an ED_{50} of ~0.5 μmol/kg, whereas phenobarbital, PCN and other compounds are far less potent. However, TCPOBOP and phenobarbital do not cause identical induction of monooxygenase activities; for example, phenobarbital treatment induced testosterone 6α-hydroxylase activity but TCPOBOP treatment depressed this enzyme activity in the B6 strain. These results, therefore, suggest that TCPOBOP modulates at least one murine cytochrome P-450 isozyme in a manner similar to phenobarbital but at a dose two to three orders of magnitude less than the latter compound and that the effect of the two compounds on other murine cytochrome P-450 isozymes may

differ (e.g. the isozyme(s) mediating 6α-hydroxylation of testosterone).

The induction of pentoxyresorufin *O*-dealkylase activity by TCPOBOP and phenobarbital in the mouse suggests that these inducers increase the microsomal content of the mouse homolog of cytochrome P-450b. This conclusion is consistent with recent reports from several laboratories using monoclonal and polyclonal antibodies to rat cytochrome P-450b which suggest that TCPOBOP and phenobarbital induce a mouse isozyme immunochemically related to cytochrome P-450b [31–33]. TCPOBOP and phenobarbital-treated mice also exhibited elevated 6β-hydroxylation of testosterone, suggesting that these compounds also induce the mouse homolog of cytochrome P-450p. The immunoquanitation experiments of Kaminsky *et al.* [34], employing anti-(rat cytochrome P-450), support the suggestion that phenobarbital increases the hepatic content of the mouse homolog of cytochrome P-450p. Additional experiments, using isozyme specific probes, are necessary to determine if TCPOBOP also induces this isozyme.

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