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Effect of the Suicide Substrate 3,5-Diethoxycarbonyl-2,6-dimethyl-4-ethyl-1,4-dihydropyridine on the Metabolism of Xenobiotics and on Cytochrome P-450 Apoproteins

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SUMMARY

Treatment of rats with the cytochrome P-450 suicide substrate, 3,5-diethoxycarbonyl-2,6-dimethyl-4-ethyl-1,4-dihydropyridine (DDEP), produced a 95% inhibition of the *in vivo* demethylation of either aminopyrine or morphine within 2 hr. One-carbon metabolism of formaldehyde or formate to carbon dioxide was not altered. DDEP also produced a time-dependent decrease in total hepatic microsomal cytochrome P-450 but had no effect on either NADPH-cytochrome *c* reductase or *p*-nitrophenol glucuronyltransferase activities up to 24 hr after administration. A rapid decrease in rat liver microsomal aniline hydroxylation and ethoxyresorufin deethylation was observed *in vitro* following DDEP administration. Although *in vitro* testosterone metabolism to 16α-, 16β-, and 2α-hydroxy metabolites was depressed profoundly by DDEP in microsomes from untreated and 3-methylcholanthrene-treated animals, 7α-hydroxylation of testosterone was much less affected. Immunochemical quantification of various microsomal cytochrome P-450 protein moieties showed that cytochromes P-450$_{\beta NF\text{-}B}$, P-450$_{UT\text{-}A}$, P-450$_{PCN\text{-}E}$, and P-450$_{PB\text{-}C}$ were decreased in hepatic microsomes from DDEP-treated rats. However, the protein moiety of cytochrome P-450$_{UT\text{-}H}$ was not diminished and the immunoreactive protein for cytochromes P-450$_{UT\text{-}F}$, P-450$_{PB\text{-}B}$, and P-450$_{ISF\text{-}G}$ was only slightly decreased. These results show that DDEP treatment leads to marked decreases in holoprotein and apoproteins of many but not all hepatic microsomal cytochrome P-450 isozymes.

The consequences of xenobiotic administration on the levels of various forms of hepatic microsomal cytochrome P-450 and the subsequent effects on drug metabolism are well known; a multitude of agents can either increase or decrease various forms of this hemoprotein. Individual forms of cytochrome P-450 are known to be induced by agents such as phenobarbital, 3-methylcholanthrene, β-naphthoflavone, and pregnenolone-16α-carbonitrile (1–3). Certain chemicals can decrease the levels of these isoenzymes by modulating the heme biosynthetic pathway and thereby affect the availability of heme and the amount of hepatic cytochrome P-450, e.g., cobaltous chloride (4, 5). Agents such as AIA can act as suicide substrates (i.e., mechanism-based inhibitors) for one or more cytochrome P-450 forms and thereby promote catabolic destruction of these cytochrome P-450 species (6, 7). Suicide substrates such as derivatives of DDC have been shown to interact rapidly with cytochrome P-450, promote the rapid destruction of hepatic microsomal cytochrome P-450, and lead to the generation *in vivo* of *N*-alkyl protoporphyrins which, in turn, inhibit the activity of mitochondrial ferrochelatase, the enzyme catalyzing the last step in heme biosynthesis (8–11). The result is a decreased total hepatic microsomal cytochrome P-450 level and a marked hepatic porphyria (12, 13).

Although the total level of hepatic microsomal cytochrome P-450 decreases following treatment of rats with certain dihydropyridines, no studies have dealt with the effects of these agents on specific forms of hepatic cytochrome P-450 or on the metabolism of drugs *in vivo*. The current study was designed to investigate the consequences of administration of the 4-ethyl derivative of DDC, DDEP, an active suicide substrate for cytochrome P-450 in rats. The metabolism of several drugs *in vivo* and xenobiotic and steroid metabolism *in vitro* were determined, and quantitative, specific antibody-directed analysis of individual forms of rat hepatic microsomal cytochrome P-450 species was performed.

Experimental Procedures

Materials

Radiochemicals. [Dimethylamine-^{14}C]aminopyrine (114 mCi/mmol), [^{14}C]-formaldehyde (14 mCi/mmol), and [*N-methyl*-^{14}C]mor-

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ABBREVIATIONS: AIA, allylisopropylacetamide; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; DDEP, 3,5-diethoxycarbonyl-2,6-dimethyl-4-ethyl-1,4-dihydropyridine.

phine hydrochloride (58 mCi/mmol) were obtained from Amersham Corp. (Arlington Heights, IL). Sodium [^{14}C]formate (40–60 mCi/mmol) and [4-^{14}C]testosterone (52 mCi/mmol) were obtained from New England Nuclear (Boston, MA).

Other chemicals and reagents. Morphine sulfate was acquired from Mallinckrodt Chemical Works (St. Louis, MO). Aminopyrine, testosterone, 3-methylcholanthrene, NADPH, and sodium phenobarbital were obtained from Sigma Chemical Co. (St. Louis, MO). Formaldehyde and sodium formate were purchased from Fisher Scientific Co. (Fair Lawn, NJ). Aniline was purchased from the Aldrich Chemical Co. (Milwaukee, WI) and was converted to the hydrochloride salt with concentrated HCl before use. 7-Ethoxyresorufin was obtained from Pierce Chemical Co. (Rockford, IL). Budget Solve and 3α70β liquid scintillation cocktails were obtained from Research Products International Corp. (Elk Grove, IL). DDEP was synthesized from propionaldehyde and ethyl acetoacetate using a method described by DeMatteis and Prior (14). The purity was verified by NMR spectroscopy. Silica gel 60 F-254 thin layer chromatographic plates (0.2 mm) were purchased from MCB Reagents (Gibbstown, NJ). They were activated at 100° for 15 min prior to use. Standards of 16α-, 6β-, and 7α-hydroxytestosterone were a gift from the Steroid Reference Collection (maintained by Dr. N. Kirk, Medical Research Council, London, U.K.).

Animals. Male Sprague-Dawley rats (200 g) were obtained from Bio-Labs (St. Paul, MN) and housed in stainless steel, wire-bottomed cages. The rats were maintained on a 12-hr light/dark cycle, and environmental temperature and humidity were rigidly controlled. Purina Rodent Chow #5001 and water were provided *ad libitum*.

Methods

Animal treatments. Rats received 3-methylcholanthrene dissolved in peanut oil (40 mg/kg, intraperitoneally) daily for 3 days. Control animals received oil alone. Phenobarbital-treated rats received sodium phenobarbital dissolved in saline (80 mg/kg, intraperitoneally) daily for 4 days. DDEP was suspended in peanut oil and injected intraperitoneally (100 mg/kg).

Preparation of microsomes. Hepatic microsomes were isolated from a 25% (wet weight/volume) liver homogenate prepared in 1.15% KCl. The homogenate was centrifuged at 9,000 × *g* for 20 min, and the resulting supernatant was centrifuged at 135,000 × *g* for 30 min to sediment the microsomal pellet. The pellet was homogenized in 10 mM Tris acetate buffer (pH 7.4) containing 20% glycerol and 1 mM sodium ethylenediaminetetraacetate.

Enzyme assays. Cytochrome P-450 content was determined by the method of Omura and Sato (15). Aniline hydroxylation was measured by the method of Imai *et al.* (16), and 7-ethoxyresorufin deethylation was determined by the method of Prough *et al.* (17). NADPH-cytochrome *c* reductase activity and *p*-nitrophenol glucuronidation were determined as described by Baron and Tephly (18) and Bock *et al.* (19), respectively. Testosterone hydroxylation was determined by the procedures of Waxman *et al* (20). In this method, the major metabolic products separated and quantified were 2α-, 7α-, 16α-, and 16β-hydroxytestosterone. Protein was determined by the method of Bradford (21).

***In vivo* studies.** Rats received [dimethylamine-^{14}C]aminopyrine at a dose of 40 μmol/kg (5 μCi/kg) or [*N-methyl*-^{14}C]morphine (morphine sulfate equivalent to 17.5 μmol of morphine base/kg, 10 μCi/kg) by intraperitoneal administration. The vehicle was saline. DDEP was administered (100 mg/kg) 2 hr before the administration of isotope. Immediately following aminopyrine or morphine injection, rats were placed in glass metabolic chambers, one rat per chamber, $^{14}CO_2$ excreted in the breath was collected and quantified as described previously (22). In certain studies, aminopyrine metabolism was followed as described by Black *et al.* (23). ^{14}C-Formaldehyde and ^{14}C-formate oxidation *in vivo* were studied as reported previously (24). Hepatic folates were determined by the method of McMartin *et al.* (25).

Quantitation of Cytochrome P-450 Apoproteins

Individual forms of cytochrome P-450 were purified from rat hepatic microsomes as reported previously by Guengerich *et al.* (3).

Antibodies to individual purified cytochromes P-450 were raised in rabbits and IgG fraction subsequently prepared using standard methods (26). The isozymic specificities of these anti-P-450 antibodies were assessed by Western blotting. In the cases where these techniques revealed a lack of monospecificity, the antisera (or IgG fractions) were further purified by cross-absorption using covalently immobilized microsomes which were enriched with the cross-reactive immunogen (27). Microsomes were solubilized with 1.5% sodium cholate and 1% Lubrol PX and then coupled covalently to Reacti-gel (Pierce Chemical Co.), using the manufacturers' recommendations. Antisera or IgG fractions were diluted in 20 mM KPi (pH 7.4) containing 0.9% (w/v) NaCl and cycled through columns of such immunoadsorbants for 16 hr at 22°. In cases where residual cross-reactivity was detected, the process was repeated with more immunoadsorbant until the desired specificity was achieved.

Quantitative estimates of individual cytochrome P-450 isoenzyme levels in liver microsomal fractions were performed by "Western blotting" as described by Guengerich *et al.* (28). A standard curve of purified cytochromes P-450 was included on each nitrocellulose sheet. In addition, an internal standard consisting of a known amount of equine liver alcohol dehydrogenase (0.2 μg; Boehringer-Mannheim, Indianapolis, IN) was added to each sample or standard prior to electrophoresis and a 1/300 dilution of rabbit anti-equine liver alcohol dehydrogenase antibody was mixed with the anti-cytochrome P-450 antiserum prior to immunochemical staining. Thus, the stained nitrocellulose sheets typically contained two bands, one at M_r = 43,000 (alcohol dehydrogenase) and one at M_r = 48,000–56,000 (cytochrome P-450). The intensities of both bands were estimated by densitometry and the ratios of the areas of the corresponding two peaks were used in all calculations. Inclusion of this internal standard helped to minimize variations in response between different gel lanes. Varying amounts of microsomal protein were assayed to identify the range of linear responses; the amount of microsomal cytochrome P-450 assayed was typically in the range of 0.1–3 pmol, depending upon the antigen and the antibody. The dilution of the primary antiserum used for staining the nitrocellulose sheets was typically in the range of 1/50–1/400. Immunoquantitations were generally performed using microsomal samples prepared from individual animals and are expressed as pmol P-450 isoenzyme/mg microsomal protein (mean ± standard deviation for three to five individuals).

Statistical Methods

For enzymatic activities, the Student's *t* test was employed to analyze for significant differences between the control and experimental groups. The one-tailed *t* test was used in the analysis of the immunoquantitations.

Results

Effects of DDEP pretreatment on drug metabolism *in vivo*. Pretreatment of rats with DDEP had a profound effect on the demethylation of aminopyrine and morphine. Fig. 1 shows that DDEP administration (100 mg/kg) 2 hr before the administration of [^{14}C]aminopyrine resulted in a 95% inhibition of the rate of $^{14}CO_2$ pulmonary excretion. Following DDEP pretreatment, aminopyrine blood levels remained elevated for prolonged time periods in agreement with a profound inhibition of *N*-demethylation (data not shown). Marked inhibition of the rate of *N*-demethylation was also found when [*N-methyl*-^{14}C]morphine metabolism to $^{14}CO_2$ was studied (Table 1) in animals treated 2 hr earlier with DDEP.

The inhibition of aminopyrine metabolism was not due to an interference with one-carbon metabolism by DDEP. The rate of ^{14}C-formaldehyde oxidation to $^{14}CO_2$ in control rats (1.41 ± 0.16% of dose/min, mean ± SE) was not significantly different compared with DDEP-pretreated animals (1.18 ± 0.18% of

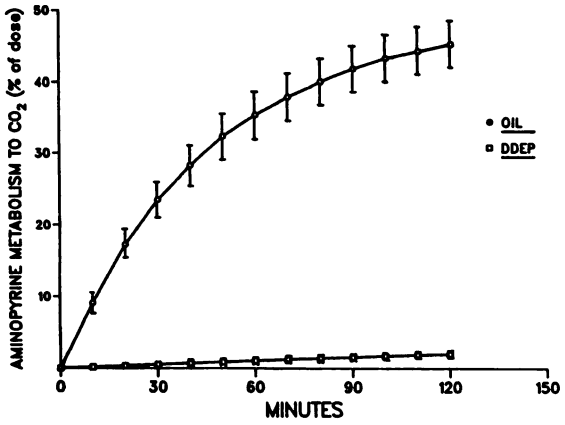

Fig. 1. Effect of DDEP pretreatment on the metabolism of [dimethylamine-^{14}C]aminopyrine to $^{14}CO_2$ *in vivo*. DDEP (100 mg/kg) was administered to rats 2 hr before the administration of [dimethylamine-^{14}C]aminopyrine to control (○——○) or DDEP-treated (□——□) rats. $^{14}CO_2$ was collected and analyzed as described in Experimental Procedures. The values represent means ± SE obtained with four animals.

TABLE 1
Effect of DDEP on [*N-methyl*-^{14}C]morphine metabolism to $^{14}CO_2$

DDEP (100 mg/kg) or peanut oil (5 ml/kg) was administered intraperitoneally 2 hr before the administration of [*N-methyl*-^{14}C]morphine (17.5 μmol/kg, intraperitoneally) and initiation of $^{14}CO_2$ collection. Each value is the mean ± SE obtained from four rats.

Treatment	Peak rate of $^{14}CO_2$ exhalation	Total $^{14}CO_2$ exhaled within 4 hr
	% of dose/min	*% of dose*
Oil	0.216 ± 0.038	15.1 ± 2.7
DDEP	0.004 ± 0.002[a]	0.5 ± 0.1[a]

[a] Value is significantly different from that for oil-treated rats ($p < 0.01$).

dose/min). Likewise, ^{14}C-formate oxidation to $^{14}CO_2$ was not affected by DDEP pretreatment (control, 0.268 ± 0.05 versus DDEP-pretreated, 0.231 ± 0.015% dose/min). Total hepatic folate, tetrahydrofolate, 5-methyltetrahydrofolate, and 10-formyltetrahydrofolate levels were unaffected by DDEP pretreatment (data not shown).

Effect of DDEP on total hepatic microsomal cytochromes P-450. Previous studies in this laboratory and others (12, 13) have shown that, after DDEP administration, there is a marked accumulation of *N*-ethylprotoporphyrin IX and a significant decrease in total microsomal cytochrome P-450. Table 2 shows the effect of DDEP on hepatic microsomal cytochrome P-450 from control, 3-methylcholanthrene-treated, and phenobarbital-treated rats. This porphyrogenic agent produced a significant decrease in cytochrome P-450 levels in each treatment group within 2 hr. Hepatic microsomal cytochrome P-450 concentrations after DDEP administration remained low for at least 24 hr. These results probably reflect both the rapid destructive, catabolic effect of this agent and the inhibition of ferrochelatase by *N*-ethylprotoporphyrin IX generated as a result of DDEP treatment.

Effect of DDEP on the *in vitro* metabolism of xenobiotics and steroids. The hydroxylation of aniline represents an activity that is catalyzed by more than one form of cytochrome P-450 (3). Pretreatment of rats with phenobarbital and 3-methylcholanthrene produced a significant increase in the hepatic microsomal aniline hydroxylase activity *in vitro* (1.32 ± 0.04 nmol/min/mg of protein and 0.61 ± 0.04 nmol/min/mg of protein, respectively) compared with microsomes from uninduced animals (0.41 ± 0.01 nmol/min/mg of protein). DDEP treatment resulted in a marked decrease in the hydroxylation of aniline in all groups (Fig. 2). Inhibition of aniline hydroxylation occurs concurrently with the decrease in levels of total microsomal cytochrome P-450. However, activity in hepatic microsomes from untreated rats was somewhat less affected by DDEP than that from either 3-methylcholanthrene- or phenobarbital-treated animals.

In contrast to aniline hydroxylation, ethoxyresorufin deethylation is largely dependent on the 3-methylcholanthrene- or β-naphthoflavone-inducible isozyme P-450$_{\beta NF\text{-}B}$ (3). 3-Methylcholanthrene pretreatment greatly increased the *in vitro* deethylation of ethoxyresorufin (2.62 ± 0.29 nmol/min/mg) compared to untreated (0.042 ± 0.001 nmol/min/mg) rat liver microsomes, a 62-fold increase. Pretreatment of animals with phenobarbital had only a slight effect (2-fold increase) on the deethylation of ethoxyresorufin (0.106 ± 0.003 nmol/min/mg). After DDEP treatments there was a rapid decrease in ethoxyresorufin deethylation in all groups (Fig. 3) which reached a nadir within 2–4 hr and remained low for 24 hr.

Testosterone hydroxylations at the 16α-, 2α-, 7α-, and 16β-positions may be used as indicators of the isozymes P-450$_{UT\text{-}A}$, P-450$_{UT\text{-}F}$, and P-450$_{PB\text{-}B}$ (3, 20). P-450$_{UT\text{-}A}$, a major isozyme in microsomes from untreated rats, catalyzes the 16α- and 2α-hydroxylation of testosterone. Phenobarbital induces

TABLE 2
Effect of DDEP on spectrally quantifiable cytochrome P-450 in rat liver microsomes

Values are expressed as nmol P-450/mg microsomal protein and represent the means ± SD obtained from 4–6 animals.

Hours after DDEP administration	Pretreatment of animals		
	None	3-Methylcholanthrene	Phenobarbital
0	0.52 ± 0.02	0.69 ± 0.07	1.51 ± 0.11
2	0.13 ± 0.01[a]	0.33 ± 0.02[a]	0.93 ± 0.01[a]
4	0.11 ± 0.01[a]	0.22 ± 0.01[a]	0.62 ± 0.08[a]
8	0.09 ± 0.01[a]	0.21 ± 0.02[a]	0.45 ± 0.01[a]
24	0.06 ± 0.01[a]	0.11 ± 0.01[a]	0.20 ± 0.10[a]

[a] Significant difference ($p < 0.05$) compared to zero time controls.

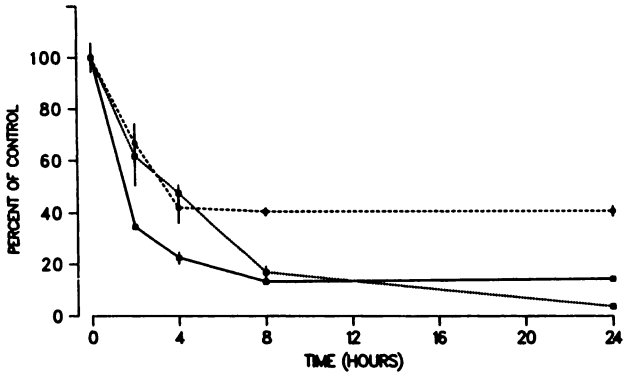

Fig. 2. Inhibition of aniline hydroxylation in rat liver microsomes by DDEP. Rats were pretreated with 3-methylcholanthrene, phenobarbital, or vehicle. Hepatic aniline hydroxylation activity was determined at various times after DDEP (100 mg/kg) administration. DDEP produced a significant decrease ($p < 0.05$) in aniline hydroxylation activity in microsomes from 3-methylcholanthrene-treated (□——□), phenobarbital-treated (■. . . .■), and untreated (●– – –●) rat livers. Values represent means ± SE obtained with 4–6 animals. Control rates are given in the text.

P-450$_{PB-B}$, the major isozyme in microsomes from phenobarbital-induced rats. P-450$_{PB-B}$ catalyzes both 16α- and 16-β-hydroxylation of testosterone. The 7α-hydroxylation is catalyzed by P-450$_{UT-F}$. P-450$_{PB-C}$, P-450$_{PCN-E}$, and P-450$_{UT-H}$ have little or no hydroxylase activity toward testosterone (20, 28).

Fig. 4 shows that 16α, 16β, and 2α-hydroxylation activities are rapidly decreased in microsomes from untreated and 3-methylcholanthrene-treated rats after treatment with DDEP, whereas the 16α- and 16β-activities are less affected in microsomes from phenobarbital-treated rats. The 7α-hydroxylation activity remains high for at least 8 hr after DDEP in microsomes from all groups of rats before declining. These data suggest that there is a differential effect of DDEP on individual cytochrome P-450 isozymes.

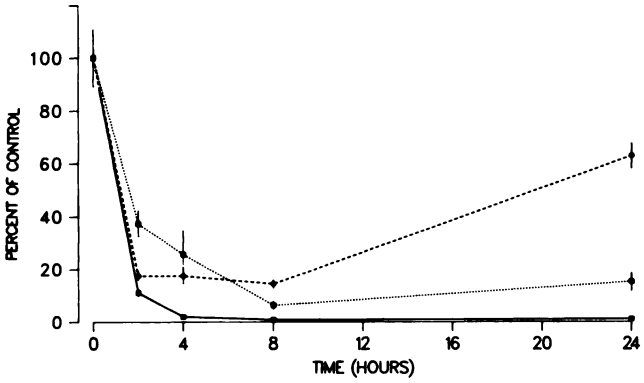

Fig. 3. Inhibition of 7-ethoxyresorufin deethylation activity was determined at various times after DDEP (100 mg/kg) administration. DDEP produced a significant decrease ($p < 0.05$) in this microsomal activity in microsomes from 3-methylcholanthrene-treated (□——□), phenobarbital-treated (■· · · ·■), and untreated (●– – –●) rat livers. Values represent means ± SE obtained with 4–6 animals. Control rates are given in the text.

Effects of DDEP on protein moieties of individual cytochrome P-450 forms determined by immunochemical quantifications. The concentration of individual cytochrome P-450 isozymes in hepatic microsomes varies depending on the pretreatment of the animal from which the microsomes were prepared. The concentrations of specific immunoreactive forms of cytochrome P-450 are shown in Table 3.

DDEP produces a decrease in the immunoreactive protein moieties of cytochrome P-450 forms, P-450$_{UT-A}$, P-450$_{PCN-E}$, and P-450$_{PB-C}$ in hepatic microsomes from rats from all treatment groups. P-450$_{\beta NF-B}$ in microsomes from 3-methylcholanthrene-treated rats is also decreased (Fig. 5). In contrast, P-450$_{UT-H}$ appears to be unaffected by DDEP in microsomes from all treatment groups, and the concentration of form P-450$_{UT-F}$ is not markedly decreased and is never below 40% of control value (Fig. 6). The P-450$_{ISF-G}$ and P-450$_{PB-B}$ concentrations in microsomes from 3-methylcholanthrene- and phenobarbital-

TABLE 3
Comparative concentrations of cytochrome P-450s in microsomes from untreated, 3-methylcholanthrene-treated, and phenobarbital-treated rats

P-450 form	Concentration of immunoreactive cytochrome P-450 (pmol/mg protein)[a]		
	Untreated	3-Methylcholanthrene	Phenobarbital
P-450$_{UT-A}$	210 ± 20	150 ± 20	160 ± 20
P-450$_{PB-B}$	very low	very low	880 ± 200
P-450$_{\beta NF-B}$	very low	1290 ± 180	very low
P-450$_{ISF-G}$	very low	570 ± 120	very low
P-450$_{PB-C}$	370 ± 50	180 ± 30	520 ± 120
P-450$_{PCN-E}$	230 ± 60	290 ± 70	540 ± 80
P-450$_{UT-F}$	110 ± 10	170 ± 10	320 ± 10
P-450$_{UT-H}$	110 ± 10	80 ± 20	60 ± 10

[a] Values represent the mean ± SD of determinations made on samples from at least three animals.

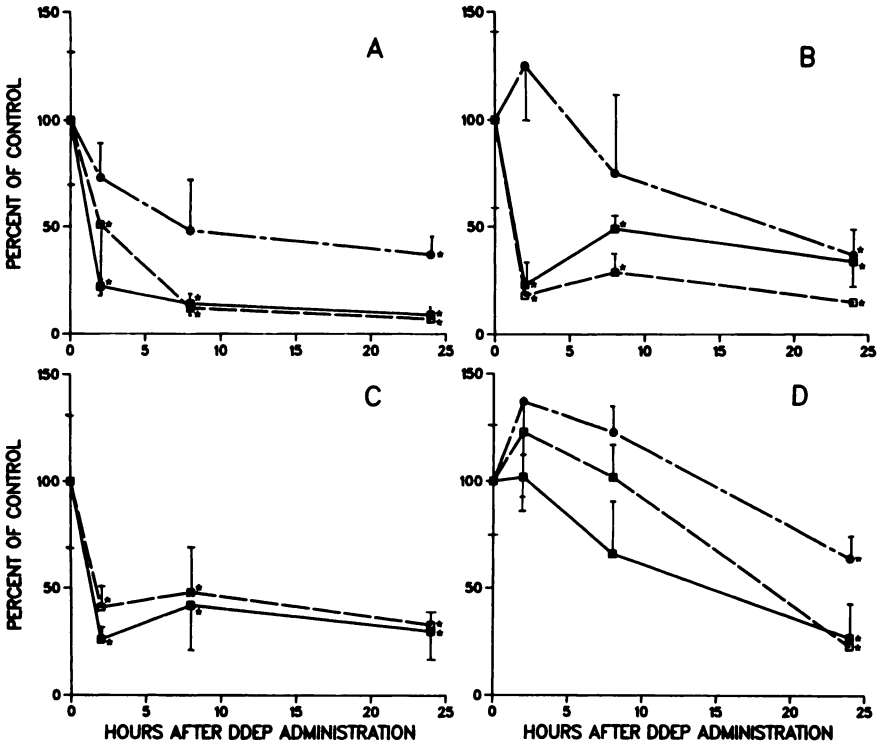

Fig. 4. Effect of the administration of DDEP on the production of various testosterone hydroxylation products in rat liver microsomes. Following administration of DDEP (100 mg/kg), the hydroxylation activities toward testosterone at the 16α (A)-, 16β (B)-, 2α (C)-, and 7α (D)-positions were determined. Control rates for uninduced (■——■) rat liver microsomes were: 2α, 0.26 ± 0.08 nmol/min/mg of protein; 16α, 0.33 ± 0.13; 16β, 0.09 ± 0.02; and 7α, 0.21 ± 0.02. Control rates for 3-methylcholanthrene (□— – –□)-pretreated rats were: 2α, 0.25 ± 0.09, 16α, 0.19 ± 0.06; 16β, 0.27 ± 0.16; and 7α, 0.43 ± 0.12. Control rates for phenobarbital (●— · —●)-pretreated rats were: 16α, 0.35 ± 0.13; 16β, 0.24 ± 0.10; and 7α, 0.16 ± 0.05. All values marked with an *asterisk* are significant by lower than the corresponding value measured at time zero, at least at the $p < 0.05$ level. Values represent means ± SD obtained with three animals.

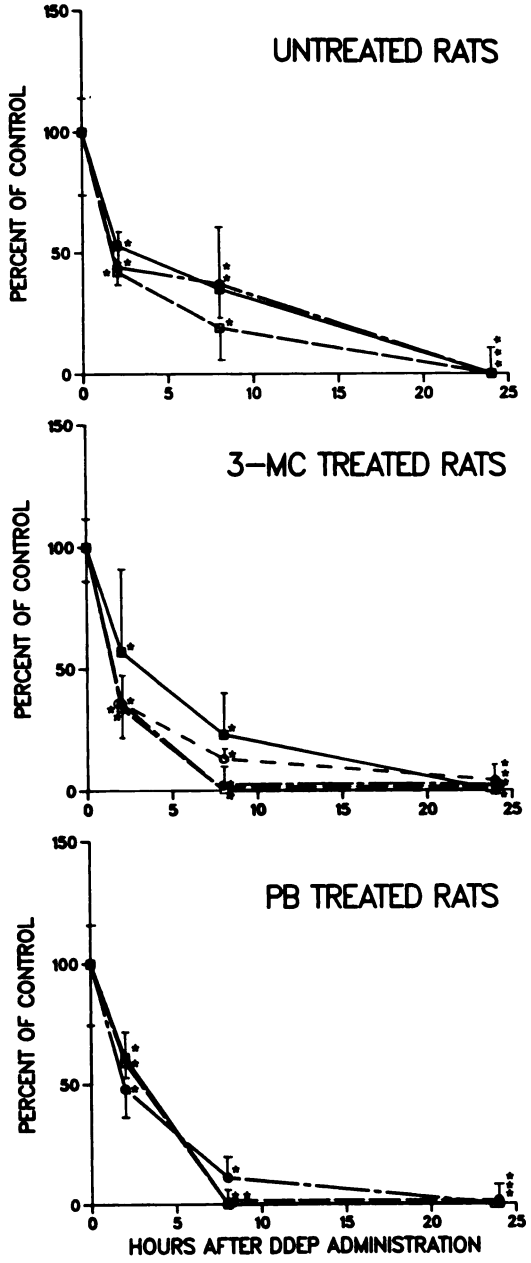

Fig. 5. Hepatic microsomal cytochrome P-450 isozymes affected strongly by DDEP treatment. Cytochrome P-450 isozymes P-450$_{\beta NF\text{-}B}$ (○---○), P-450$_{UT\text{-}A}$ (■——■), P-450$_{PB\text{-}C}$ (●—·—●), and P-450$_{PCN\text{-}E}$ (□—·—□) were quantitated in rat liver microsomes at various times after DDEP (100 mg/kg) administration to phenobarbital (*PB*)- and 3-methylcholanthrene (*3-MC*)-induced and untreated rats. Values represent means ± SD of measurements made with three animals. Control values are listed in Table 3. All values marked with an *asterisk* are significantly lower than the corresponding values measured at time zero, at least at the $p < 0.05$ level.

treated rats, respectively, are also less affected than those reported in Fig. 5.

Discussion

Treatment of rats with DDEP leads to a series of events which includes reaction of the dihydropyridine with hepatic microsomal cytochrome P-450, a transfer of the 4-ethyl group to the prosthetic heme group, and a catabolic destruction of the heme protein (13). This results in a rapid decrease in spectrally quantifiable total hepatic microsomal cytochrome P-450 and the formation of *N*-ethylprotoporphyrin IX. The latter metabolite inhibits the activity of mitochondrial ferrochelatase, the enzyme mediating the last step in heme biosynthesis, the result being an induced porphyria.

As a consequence of the destruction of the hemoprotein, a profound inhibition of enzymatic activities toward substrates, whose metabolism is catalyzed by several cytochrome P-450

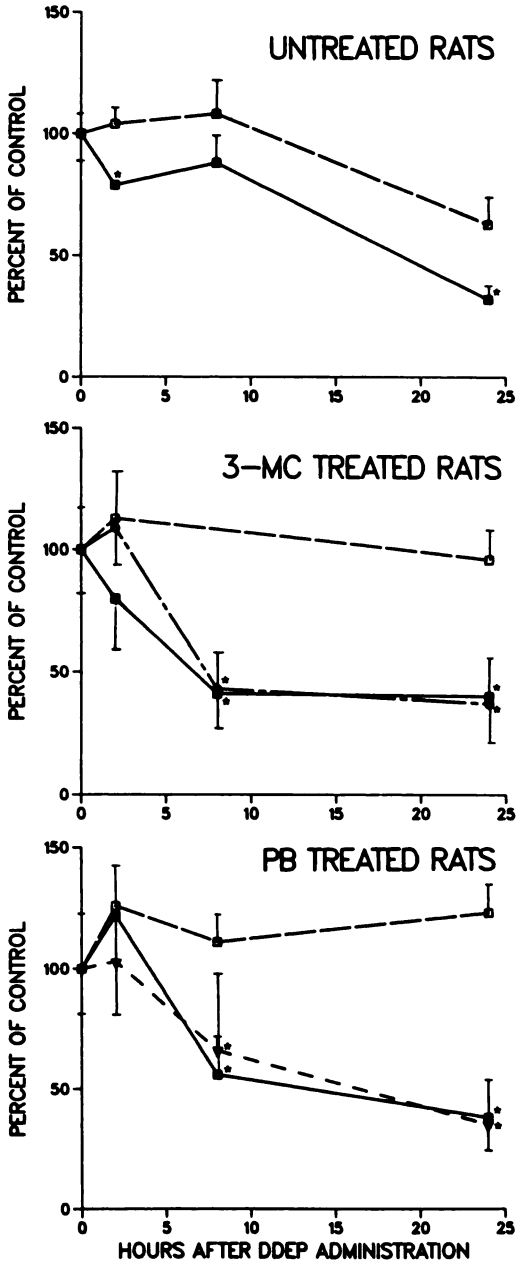

Fig. 6. Hepatic microsomal cytochrome P-450 isozymes less affected by DDEP treatment. Cytochrome P-450 isozymes P-450$_{ISF\text{-}G}$ (○—·—○), P-450$_{UT\text{-}H}$ (□—·—□), P-450$_{UT\text{-}F}$ (■——■), and P-450$_{PB\text{-}B}$ (▲---▲) were quantitated in rat liver microsomes at various times after DDEP administration (100 mg/kg) to phenobarbital (*PB*)- and 3-methylcholanthrene (*3-MC*)-induced and untreated rats. Values represent means ± SD of measurements made with three animals. Control values are listed in Table 3. All values marked with an *asterisk* are significantly lower than the corresponding values measured at time zero, at least at the $p < 0.05$ level.

isozymes, takes place both *in vivo* and *in vitro*, following DDEP treatment. The *N*-demethylation *in vivo* of aminopyrine and morphine and the hydroxylation of aniline *in vitro* are rapidly decreased following DDEP treatment.

Ethoxyresorufin deethylation, which is linked to P-450$_{\beta NF\text{-}B}$, is also rapidly diminished *in vitro* in microsomes from DDEP-treated rats; however, the 7α-hydroxylation of testosterone is not altered significantly until 24 hr following DDEP treatment. Other hydroxylations of testosterone, such as 16α-, 16β-, and 2α-hydroxylations, are more markedly affected in untreated and 3-methylcholanthrene-induced rats, compared to the 7α-hydroxylation.

Other hepatic microsomal activities, such as NADPH-cytochrome *c* reduction and *p*-nitrophenol glucuronidation are not changed (data not shown) over the time course of these experiments (24 hr after DDEP). In addition, we have found that DDEP has no effect on the oxidation of either formaldehyde or formate to carbon dioxide, substances whose metabolism is linked to the folate pathway (24), and has no effect on the steady state levels of hepatic folates. Also, DDEP did not display general hepatotoxicity as determined by examination of several transaminase levels at doses used in this experiment, and histological examination of the livers showed no abnormalities following DDEP doses of 100 mg/kg.

The DDEP treatment leads to a decrease in immunoreactive protein moieties of a number of cytochrome P-450 isozymes. The apoprotein of P-450$_{BNF\text{-}B}$ is particularly affected and markedly decreased and likewise are the protein moieties of P-450$_{UT\text{-}A}$, P-450$_{PCN\text{-}E}$, and P-450$_{PB\text{-}C}$, following DDEP treatment. In contrast, P-450$_{UT\text{-}H}$ apoprotein is not affected at all. Interestingly, there is an apparent correlation between the effect of DDEP on 7α-hydroxylation of testosterone which is mediated by P-450$_{UT\text{-}F}$ and the effect on the P-450 apoprotein. Also, in microsomes from phenobarbital-treated rats the effect of DDEP on 450$_{PB\text{-}B}$ appears to correlate with the effect on the 16α- and 16β-hyroxylations of testosterone.

Although immunoreactions were only carried out on certain selected but dominant isozymes, it is evident that DDEP treatment enriches the microsomes in P-450$_{UT\text{-}F}$ and P-450$_{UT\text{-}H}$, relatively speaking. This may explain the shift of the maximum of the reduced CO-complex toward longer wavelengths, which has been observed previously after treatment with DDEP (13). The wavelength maxima of P-450$_{UT\text{-}F}$ and P-450$_{UT\text{-}H}$ are 452 and 449 nm, respectively (20, 29). However, the 4 methyl analog of DDEP, DDC, does not cause a shift in the wavelength maximum although total hepatic microsomal cytochrome P-450 levels are lowered after treatment with DDC (13). Another suicide substrate, AIA, preferentially destroys cytochrome P-450 isozymes associated with phenobarbital induction (7, 30) but has little effect on isozymes inducible by 3-methylcholanthrene (30). This suggests that it might be possible to selectively deplete the microsomes of specific cytochrome P-450 isozymes by treatment with xenobiotics, classified as suicide substrates. Since many drugs and environmental xenobiotics are suicide substrates for cytochrome P-450 (31), and other compounds among these groups of agents are inducers of cytochrome P-450, it is feasible to alter the composition of cytochrome P-450 in the liver with the selective use of inducers and suicide substrates.

The mechanism of destruction of specific cytochrome P-450 apoproteins cannot be derived unambiguously from these experiments. The decrease in apoprotein follows the decreases in enzymatic activities and spectrally quantifiable cytochrome P-450, suggesting that the loss of the heme prosthetic group promotes catabolic destruction of the apoprotein or that the protein moities are directly attacked by metabolites of DDEP. AIA also promotes the loss of heme from cytochrome P-450 but leaves at least some apoprotein intact, since it is possible to partly restore some enzymatic activities and CO-complex by hemin pretreatment *in vivo* and by hemin incubation *in vitro* (32). However, hemin incubation of liver homogenates from DDEP-treated rats following the procedures of Bornheim *et al.* (32) did not yield any restoration of the CO-complex in our experiments. The generation of ethyl radicals after interaction of DDEP with hepatic microsomes has been demonstrated by Augusto *et al* (33). This type of agent may be involved in the destruction of the protein moieties of cytochrome P-450 isozymes.

DDEP does not affect certain forms of cytochrome P-450 to the degree that it perturbs others. This might be because DDEP is not a substrate for those forms not altered, or because these isozymes are so situated in the endoplasmic membrane that protection against DDEP is afforded by endogenous substrates in the membrane. Finally, one cannot rule out selective endogenous substrate protection as a potential protective mechanism for certain isozymes.

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