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Induction of hepatic microsomal cytochrome P-450 and associated monooxygenases by pentamethylbenzene in the rat

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Cytochrome P-450, the terminal oxidase of the hepatic microsomal election transport system, and a variety of mixed-function oxidase (MFO) activities are inducible following treatment of animals with a wide variety of zenobiotics [1–3]. Multiple forms of mammalian cytochrome P-450 have also been identified and characterized by enzymatic, immunological, and biochemical techniques [2–6].

Insects, like mammals, have been shown to possess inducible MFO systems [7–9], and studies in this laboratory with the southern armyworm (*Spodoptera eridania*) have established that MFO activity in the larval midgut tissues of this insect is highly responsive to dietary treatment with pentamethylbenzene (PMB) and other alkylbenzenes [8]. Subsequent studies have shown that, of the several non-insect species tested, only MFO activities of the rat were affected appreciably by PMB administration. This communication reports some of the unusual effects of PMB administration on rat hepatic MFO activities.

Male Sprague–Dawley-derived rats (200 mg), obtained from Blue Spruce Farms, Altamont, NY, were injected i.p. for 3 successive days with either phenobarbital (PB, 100 mg/kg) in 0.9% saline), 3-methylcholanthrene (MC, 20 mg/kg in corn oil), or pentamethylbenzene (PMB, 2.5 g/kg in corn oil). Control animals received the appropriate vehicles only, and no significant differences were observed between MFO values in microsomes from control and untreated rats. Animals (four per treatment group) used in the dose–response study received a single injection of the appropriate concentration of each inducer, and microsomes were prepared 24 hr later. In all cases, microsomes were prepared as previously described [10] and protein was determined by the method of Lowry *et al.* [11], using bovine serum albumin as a standard. Cytochrome P-450 was measured according to the method of Omura and Sato [12] using an extinction coefficient of 91 mM^{-1} cm^{-1} for the CO-ferrocytochrome P-450 spectral complex. Aminopyrene *N*-demethylase (APDM) activity was measured by the liberation of formaldehyde [13], and aniline *p*-hydroxylase (APH) activity was assayed as previously described [14]. For APDM and APH activities, incubation contained cofactors as described previously and consisted of 16 μmoles of glucose-6-phosphate (G-6-P), 2 units of G-6-P dehydrogenase and 2.5 μmoles of NADP. Aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase (AHH) activity was measured by the direct fluorometric assay of Yang and Kicha [15], and 7-ethoxycoumarin *O*-deethylase (7EC) activity was determined according to the method of Ullrich and Weber [16]. The inhibitors α-naphthoflavone (ANF) and 1-phenylimidazole (PI) were employed *in vitro* at final concentrations of 10^{-5} M and 10^{-4} M respectively.

Benzo[*a*]pyrene, PMB and ANF were purchased from the Aldrich Chemical Co., Milwaukee, WI. PB, MC and NADPH were from the Sigma Chemical Co., St. Louis, MO; G-6-P, G-6-P dehydrogenase and NADP were from Boehringer Mannheim Biochemicals, Indianapolis, IN; and PI was obtained from Trans World Chemicals, Washington, DC. All other chemicals were of analytical reagent grade.

The effects of pretreating rats with PMB were compared with those resulting from PB- and MC-treatment with respect to hepatic microsomal cytochrome P-450 levels and associated oxidase activities. Dose–response relationships from a single administration of the inducer, as well as the effects of repeated dosing, were studied.

Dose–response curves for the effects of PB, MC and PMB of 7EC and AHH activities (Fig. 1) clearly showed that PMB was not an effective inducer of AHH activity.

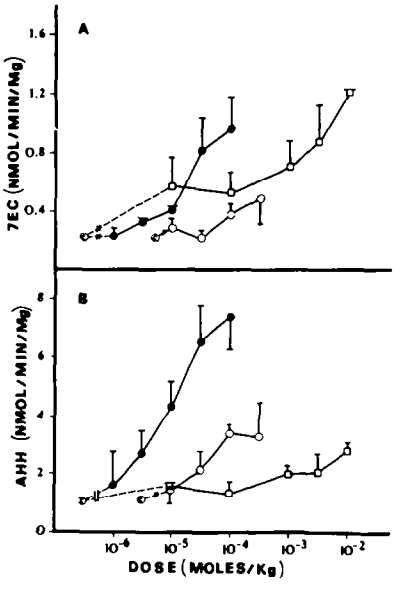

Fig. 1. Dose-response curves for the induction of 7EC (A) and AHH (B) activity in the rat. Enzyme assays and treatments with vehicle alone (C), MC (●——●), PB (○——○), and PMB (□——□) were described in the text. Each value represents the mean ±S.D. of at least four animals.

Table 1. Effects of *in vivo* treatment of rats with MC, PB, and PMB on hepatic microsomal cytochrome P-450 levels and oxidase activity*

Inducer	AHH	7EC	APDM	APH	P-450 (λ_{max} nm)
None	0.95 ± 0.28	0.26 ± 0.14	2.55 ± 0.24	0.24 ± 0.01	0.87 ± 0.01 (450)
MC	8.48 ± 0.84	0.64 ± 2.15	2.16 ± 0.08	0.52 ± 0.02	0.94 ± 0.09 (448.5)
PB	2.18 ± 0.90	0.84 ± 0.08	6.63 ± 0.62	0.41 ± 0.01	1.42 ± 0.10 (450)
PMB	1.91 ± 0.46	1.83 ± 0.45	4.80 ± 0.26	0.48 ± 0.09	1.45 ± 0.37 (449)

* Activities of each assay are expressed as the mean ± S.D. of three to seven assays. Activity units are: AHH (nmoles benzo[*a*]pyrene metabolized/min/mg protein), 7EC (nmoles umbelliferone produced/min/mg protein), APDM (nmmoles formadehyde produced/min/mg protein), and APH (nmoles *p*-aminophenol produced/min/mg protein); P-450 levels are expressed as nmoles/mg protein.

causing only minimal effects at doses as high as 10^{-2} moles/kg. PMB, however, proved to be an effective inducer of 7EC activity and, indeed, at equivalent dose levels was somewhat more active than PB.

The effects of repeated dose studies with PMB, PB and MC are shown in Table 1. At the high dose level of 2.5 g/kg, for each of 3 consecutive days, PMB increased microsomal cytochrome P-450 and AHH levels to those measured in microsomes from rats pretreated with PB (100 mg/kg for each of 3 days); APH activity was enhanced 2-fold following PMB treatment, an increase similar to that observed following treatment with MC and somewhat greater than that caused by PB. The most dramatic effect of PMB treatment was in 7EC activity where an approximately 7-fold induction was observed compared with increases of 3.2- and 2.5-fold in microsomes from PB- and MC-induced animals. PMB was more effective than MC but less effective than PB as an inducer of APDM activity. Previous studies [9] have established that PMB is inactive as an inducer of *p*-chloro-*N*-methylaniline demethylase (CMAD), a reaction markedly inducible by PB. These data suggest that PMB is behaving primarily as a "PB-like" inducing agent but that it possesses some rather unusual properties.

Since recent studies in this laboratory have clearly established that PI and ANF are selective inhibitors of reactions catalyzed by cytochromes P-450 (PB-type) and cytochromes P-448 (MC-type), respectively [17, 18], it was considered of interest to evaluate the effects of these two inhibitors on AHH activity induced by MC, PB, and PMB. The results (Table 2) clearly show that AHH activity in microsomes from PMB-induced rats was inhibited by PI and was stimulated by ANF. This was very similar to the results obtained with PB-induced microsomes and the opposite of those observed in MC-induced microsomes where ANF was strongly inhibitory. These data, as well as those obtained previously in this laboratory [9], suggest that PMB is an atypical "PB-like" inducer of cytochrome P-450 in rats.

Table 2. Effects of cytochrome P-450 inhibitors on MC-, PB- and PMB-induced AHH activity

Inducer	Inhibitor	AHH activity*	Percent of uninhibited activity
None		0.95 ± 0.21	
MC		8.48 ± 0.84	100
	PI (10^{-4} M)	8.47 ± 1.08	100
	ANF (10^{-5} M)	3.09 ± 1.24	36
PB		2.17 ± 0.90	100
	PI (10^{-4} M)	0.37 ± 0.27	17
	ANF (10^{-5} M)	2.33 ± 0.85	
PMB		1.91 ± 0.46	100
	PI (10^{-4} M)	0.40 ± 0.12	21
	ANF (10^{-5} M)	3.89 ± 0.65	204

* Expressed as nmoles benzo[*a*]pyrene metabolized/min/mg protein; values means ± S.D. of four to seven assays.

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