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SPECIES DIFFERENCES IN STIMULATION OF INTESTINAL AND HEPATIC MICROSOMAL MIXED-FUNCTION OXIDASE ENZYMES

CRISTOBAL L. MIRANDA* and RAJENDRA S. CHHABRA
Environmental Biology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, U.S.A.

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Abstract—The effect of intraperitoneal (i.p.) administration of phenobarbital (PB) or 3-methylcholanthrene (3-MC) on some mixed-function oxidase (MFO) enzymes was studied in small intestine and liver of male rats, mice, guinea pigs and rabbits. PB treatment enhanced intestinal and 7-ethoxycoumarin deethylase activities in the mouse and rat, whereas benzo[*a*]pyrene hydroxylase (AHH) activity was increased only in the mouse. Ethylmorphine demethylase and aniline hydroxylase activities in small intestine were not stimulated by PB in any species. Administration of 3-MC increased the activity of intestinal AHH in rat, mouse and guinea pig, but intestinal 7-ethoxycoumarin deethylase activity was elevated only in the rat. The guinea pig and mouse intestinal ethoxycoumarin deethylase activity was inhibited by 3-MC treatment. None of the enzymes tested in rabbit intestine was induced by PB or 3-MC. The hepatic activities of ethylmorphine demethylase, aniline hydroxylase, 7-ethoxycoumarin deethylase and AHH, and the cytochrome P-450 content were increased by PB in all species. In contrast, 3-MC enhanced hepatic aniline hydroxylase and AHH activities in rats, mice and guinea pigs, and hepatic 7-ethoxycoumarin deethylase activity in mice and rats. In rabbits, these hepatic enzymes were inhibited by 3-MC pretreatment. The hepatic cytochrome P-450 absorption spectra was shifted to 448 nm in all species. These results suggest that there are differences in induction of intestinal and hepatic MFO enzymes which are influenced by the type of inducing agent, substrate and animal species used.

The importance of extrahepatic metabolism of foreign chemicals has become increasingly evident [1–3]. Recent reports from our laboratory have shown the distribution of intestinal mixed-function oxidase (MFO) enzymes in various animal species [2, 3] and found the rabbit to be the species having the highest rates of MFO enzyme activities in small intestine [3]. Further studies of biochemical properties of some MFO enzymes in rabbit small intestine generally showed similarities between liver and intestinal enzymes [4].

Induction of intestinal MFO enzymes in rats and mice by a wide variety of chemicals is well documented [5–29]. However, there is no systematic study in the literature describing differences or similarities in stimulation of hepatic and intestinal MFO enzymes in various species. The objective of the present investigation was to determine whether there are tissue- and species-specific differences in the stimulation of microsomal MFO enzymes by foreign chemicals.

MATERIALS AND METHODS

Animals. Male rats of the CD strain, weighing 250–300 g, male mice of the CD1 strain, weighing 25–30 g, and male guinea pigs (Hartley), weighing 300–400 g, were purchased from the Charles River Breeding Laboratories (Wilmington, MA). Male New Zealand white rabbits, weighing 2.5–3 kg, were obtained from Dutchland Laboratory Animals (Denver, PA). The rats and mice were housed in plastic boxes (four per box) with Absorb-Dri bedding, and the guinea pigs and rabbits were placed individually in stainless steel cages with wire mesh bottoms. All animals were kept in a 12-hr light/dark cycle under controlled temperature (70°F ± 2) and humidity (50% ± 10). Mice and rats were fed NIH 31 diet, and rabbits and guinea pigs were given NIH Feed A. The animal feed was monitored regularly for chlorinated pesticides and estrogen levels.

Materials. Phenobarbital sodium and 3-MC were purchased from the Mallinckrodt Chemical Works (St. Louis, MO) and the Sigma Chemical Co. (St. Louis, MO), respectively. All other chemicals used in this study were obtained from sources reported previously [4].

Treatment. PB or 3-MC was administered intraperitoneally to various animals, and dose levels were selected which, in general, resulted in maximum stimulation of hepatic or intestinal microsomal MFO enzymic activities. PB, dissolved in physiological saline, was injected i.p. daily for 3 days at the following dose levels: 25 mg/kg for rabbits, 80 mg/kg for rats and guinea pigs, and 100 mg/kg for mice. The volume of solution injected was 10 ml/kg body weight for mice, 2 ml/kg for rats and guinea pigs, and 1 ml/kg for rabbits. Control animals received similar volumes of saline only. 3-MC was injected

* Present address: Department of Agricultural Chemistry, Oregon State University, Corvalis, OR 97331, U.S.A.

i.p. daily for 3 days in mice at a dose level of 100 mg in 10 ml corn oil per kg body weight; in rats and guinea pigs, 25 mg in 2 ml corn oil per kg body weight; and in rabbits, 25 mg in 1 ml corn oil per kg body weight. Corresponding control animals received corn oil only. All animals were killed by cervical dislocation following light anesthesia by CO_2 approximately 24 hr after the last treatment.

Preparation of microsomes. Immediately after the animals were killed, the liver and a segment of the small intestine (proximal 12 inches for rats, mice and guinea pigs, and proximal 24 inches for rabbits) were dissected. The small intestine was flushed three times with ice-cold physiological saline and then transferred to ice-cold KCl-Hepes (1.15% KCl containing 1.5 mM *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid adjusted to pH 7.4 with KOH). The small intestines from three to six animals were pooled except in rabbit. The livers, after removal of the gall bladder (non-existent in rats), were placed in beakers containing ice-cold KCl-Hepes. For mice, four livers were pooled per sample to obtain enough microsomal protein for enzymic assays, whereas in other species individual liver yielded enough microsomal protein for enzymic assays. The livers of all species and the small intestines of rats, mice and guinea pigs were minced before homogenization. The use of whole intestinal tissue for the preparation of the microsomal fraction in mice, rat and guinea pig was based on the fact that we did not find any difference between the specific activities of MFO enzymes in microsomal fractions prepared from whole intestinal homogenates and those from the mucosa scraped according to the method of Hoensch *et al.* [30]. For the preparation of rabbit intestinal microsomes, the homogenates of mucosal scrapings were used since this preparation gave higher yields of microsomal protein than the microsomal preparation from the homogenates of the whole intestinal segment, thus eliminating the need of tissue pooling. However, MFO enzymic activities per mg of microsomal protein were similar in both preparations. The microsomal fractions were prepared by differential centrifugation as described previously [3].

Enzyme assays. The activities of various enzymes were determined in microsomal fractions as follows: ethylmorphine demethylase activity was measured according to the procedure of Nash [31]; aniline hydroxylase was determined as described by Chhabra *et al.* [32]; AHH activity was determined by the method of Wattenberg *et al.* [5] and Hansen and Fouts [33]; and 7-ethoxycoumarine deethylase activity was estimated according to the procedure described by Ullrich and Weber [34] and modified by Pohl *et al.* [35]. All enzyme activities were expressed as nmoles product formed per mg of microsomal protein per min except for AHH. AHH activities were expressed as relative fluorescence units per mg of microsomal protein per min. Microsomal protein was determined by the method of Lowry *et al.* [36]. Cytochrome P-450 content was calculated using an extinction coefficient of 91 cm^{-1} mM^{-1} between 450 and 490 nm [37] of CO difference spectrum of dithionite reduced microsomes. Cytochrome P-450 contents in mice and rat intestine were not determined due to the reasons described in a

Table 1. Activities of ethylmorphine demethylase, aniline hydroxylase, AHH and 7-ethoxycoumarin deethylase in the small intestine of various animal species treated with phenobarbital (PB)*

Species	Tissue	Ethylmorphine demethylase		Aniline hydroxylase		AHH		7-Ethoxycoumarin deethylase	
		Control	PB	Control	PB	Control	PB	Control	PB
Rat	Liver	11.1 ± 1.4	17.0 ± 0.8†	1.4 ± 0.2	2.3 ± 0.2†	6182 ± 301	3816 ± 307†	0.50 ± 0.11	2.79 ± 0.15†
	Intestine	Trace	Trace	Trace	Trace	33 ± 9	21 ± 5	0.049 ± 0.008	0.123 ± 0.019†
Mouse	Liver	11.4 ± 0.7	34.8 ± 0.8†	3.3 ± 0.2	5.0 ± 0.2†	890 ± 74	2535 ± 313†	1.60 ± 0.19	6.19 ± 0.25†
	Intestine	Trace	Trace	Trace	Trace	32 ± 9	153 ± 23†	0.064 ± 0.012	0.260 ± 0.023†
Guinea pig	Liver	4.0 ± 0.1	15.5 ± 1.7†	1.5 ± 0.1	8.9 ± 1.0†	2920 ± 182	8291 ± 587†	1.05 ± 0.11	5.30 ± 0.61†
	Intestine	1.58 ± 0.15	1.79 ± 0.14	0.35 ± 0.03	0.23 ± 0.03	1472 ± 100	1257 ± 99	0.092 ± 0.010	0.150 ± 0.030
Rabbit	Liver	2.7 ± 0.5	5.9 ± 0.5†	1.0 ± 0.2	2.1 ± 0.2†	915 ± 164	1872 ± 409	0.67 ± 0.10	3.7 ± 0.5†
	Intestine	0.39 ± 0.06	0.44 ± 0.09	0.33 ± 0.05	0.27 ± 0.01	457 ± 120	410 ± 34	0.059 ± 0.020	0.041 ± 0.007

* Mean microsomal enzyme activity ± S.E. (N = 4). In some instances, tissues were pooled from a number of animals. N represents data from individual animals or different pooled samples. See text for details.
† $P < 0.05$.

previous publication [3]. The preparation of rat or mice intestinal microsomal fraction according to the methods of Hoensch *et al.* [30] and Stohs *et al.* [22] did not resolve the problems encountered in determination of cytochrome P-450. The estimation of cytochrome P-450 in villous tip cells of rat intestine (prepared according to Ref. 21) was also attempted, but rapid clumping of cells prevented us from obtaining meaningful cytochrome P-450 difference spectra.

Statistics. Student's *t*-test [38] was used in analyzing the data, and results were considered significant if $P < 0.05$.

RESULTS

Table 1 shows the effects of PB administration on hepatic and intestinal MFO enzyme activities in four species. All hepatic microsomal MFO activities were enchanced in phenobarbital-pretreated animals except in rabbits where hepatic AHH activities were not increased significantly. The intestinal MFO enzyme activities were not altered in guinea pig and rabbit. In mice, both AHH and 7-ethoxycoumarin deethylase activities were increased, but in rat intestine only 7-ethoxycoumarin metabolism was stimulated. The stimulation of hepatic MFO enzyme activities was accompanied by a concomitant increase in cytochrome P-450 content (Table 3). The cytochrome P-450 contents in rabbit and guinea pig intestine were not affected by PB treatment.

Table 2 shows the effects of 3-MC administration on MFO enzyme activities in small intestine and liver. The activities of all the hepatic enzymes studied were increased in mice, rat and guinea pig, except 7-ethoxycoumarin deethylase activity which was not altered in guinea pig liver. In rabbit liver, hepatic AHH and 7-ethoxycoumarin deethylase were decreased by 3-MC treatment. Hepatic cytochrome P-450 content was increased in all species (Table 3). Cytochrome P-450 absorption spectra were shifted to 448 nm in liver microsomes of all species. Intestinal AHH and 7-ethoxycoumarin deethylase activities were increased in rat and mice. In guinea pig, only AHH activities were increased, while there was a decrease in 7-ethoxycoumarin deethylase activities. Rabbit intestinal MFO enzymes were not affected by 3-MC treatment. Intestinal cytochrome P-450 contents were not altered in any species studied.

DISCUSSION

Species differences in stimulation of intestinal MFO enzymes have not been studied extensively. Previous studies on stimulation of intestinal MFO enzymes have been confined mainly to rats [5–23] and mice [24–29]. Some MFO enzyme activities which were stimulated by xenobiotics in the small intestine were AHH in rats [5–10, 23] and mice [8, 25], 7-ethoxycoumarin deethylase in rats [21, 23] and mice [27–29, 34], phenacetin demethylase in rats [23], and NADPH-cytochrome *c* reductase in mice [27]. Reports on stimulation of some enzymes such as hexobarbital oxidase [19, 39], biphenyl 2-hydroxylase, and biphenyl 4-hydroxylase [14, 21] are conflicting. In our study, the enzymes which were enchanced in the small intestine were AHH and 7-ethoxycoumarin deethylase, and depended on the species and the type of inducer. We were unable to enhance intestinal MFO activities in rabbit with either PB or 3-MC pretreatment. In rabbit, PB stimulated only the liver MFO enzymes, whereas 3-MC had no effect on either liver or intestinal MFO activities. Instead, 3-MC treatment decreased the activities of hepatic aniline hydroxylase, 7-ethoxycoumarin deethylase and AHH, despite the increase in hepatic cytochrome P-450 content. This has been reported by other workers as well [40].

Since most of the drugs or environmental agents are ingested, the inducers were given orally as well. The data from preliminary experiments suggested that oral or i.p. administration of 3-MC or PB generally did not affect the stimulation of MFO activities in the intestine of the animals studied (data not shown). Therefore, the route of administration of the inducing agent was not the reason for not observing stimulation of MFO activities in rabbit intestine.

This and other studies show that stimulation of MFO is tissue specific. For instance, Feuer *et al.* [39]

Table 2. Activities of aniline hydroxylase, benzo[*a*]pyrene hydroxylase and 7-ethoxycoumarin deethylase in the small intestine of various animal species treated with 3-methylcholanthrene (3-MC)*

Species	Tissue	Aniline hydroxylase		AHH		7-Ethoxycoumarin deethylase	
		Control	3-MC	Control	3-MC	Control	3-MC
Rat	Liver	1.4 ± 0.1	2.0 ± 0.1†	7164 ± 719	11,199 ± 609†	0.43 ± 0.06	6.71 ± 0.46†
	Intestine	Trace	Trace	40 ± 13	646 ± 137†	0.026 ± 0.010	0.320 ± 0.050†
Mouse	Liver	2.5 ± 0.1	3.8 ± 0.2†	919 ± 84	4716 ± 602†	0.70 ± 0.09	2.64 ± 0.27†
	Intestine	Trace	Trace	50 ± 14	258 ± 64†	0.040 ± 0.008	0.010 ± 0.005†
Guinea pig	Liver	2.0 ± 0.2	4.3 ± 0.1†	1266 ± 62	3601 ± 65†	1.39 ± 0.17	1.25 ± 0.07
	Intestine	0.27 ± 0.02	0.26 ± 0.02	408 ± 43	980 ± 207†	0.102 ± 0.013	0.059 ± 0.017†
Rabbit	Liver	1.0 ± 0.1	0.9 ± 0.1	2743 ± 478	1635 ± 480†	0.57 ± 0.04	0.36 ± 0.04†
	Intestine	0.47 ± 0.15	0.32 ± 0.06	383 ± 95	316 ± 66	0.046 ± 0.009	0.044 ± 0.003

* Mean microsomal enzyme activity ± S.E. (N = 4). In some instances, tissues were pooled from a number of animals. N represents data from individual animals or different pooled samples. See text for details.

† $P < 0.05$.

Table 3. Cytochrome P-450 content of microsomes from small intestine and liver of various animal species treated with phenobarbital (PB) or 3-methylcholanthrene (3-MC)*

Species	Small intestine				Liver			
	Control	PB	Control	3-MC	Control	PB	Control	3-MC
Rat	ND†	ND†	ND†	ND†	0.83 ± 0.08	1.74 ± 0.08‡	1.34 ± 0.08	2.23 ± 0.09‡
Mouse	ND†	ND†	ND†	ND†	1.65 ± 0.06	2.73 ± 0.23‡	1.06 ± 0.02	1.81 ± 0.05‡
Guinea pig	0.222 ± 0.02	0.231 ± 0.03	0.26 ± 0.014	0.26 ± 0.004	1.13 ± 0.08	1.59 ± 0.14‡	1.15 ± 0.10	1.76 ± 0.13‡
Rabbit	0.41 ± 0.06	0.40 ± 0.04	0.54 ± 0.12	0.34 ± 0.03	1.88 ± 0.19	3.19 ± 0.10‡	1.99 ± 0.26	3.20 ± 0.42‡

* Values are expressed in nmoles of cytochrome P-450 per mg of microsomal protein ± S.E.
† Not determined (see text).
‡ $P < 0.05$.

observed that PB increased the activities of aniline hydroxylase and aminopyrine demethylase in rat liver but not in small intestine. In our study, all MFO enzymic activities were stimulated in liver by PB or 3-MC (except in the case of rabbit), but the alteration in metabolism in the small intestine was limited to certain drug substrates and species. The magnitude of induction of MFO activities was generally lower in small intestine than in liver. The differences observed in stimulation of MFO enzymes between liver and small intestine suggest the possibility that factors regulating MFO activity and mechanisms of stimulation of MFO enzymes in liver may be different from those in small intestine.

Failure to observe stimulation of MFO enzymes in rabbit intestine may have two possible causes: (a) New Zealand rabbit intestinal enzymes may be genetically non-responsive to PB or 3-MC, or (b) the intestinal enzymes may have been at an induced state prior to the initiation of the study because of the possible presence of exogenous inducers in rabbit feed. Wattenberg [41] has proposed that much of the AHH activity in the rat small intestine is due to exogenous inducers present in crude diets of the rat. However, recent studies in our laboratory on the effect of crude diets vs semi-purified diets on rabbit intestinal MFO activities show that, unlike the rat, the feeding of semi-purified diets did not alter rabbit intestinal MFO enzyme activities. The details of this study will be reported later.

In conclusion, though intestinal mixed-function oxidase enzymes are similar to liver enzymes with respect to their biochemical properties (3,4,21,23,29), their response to pretreatment with inducing agents such as PB or 3-MC is different. This warrants a comprehensive study of factors regulating intestinal MFO enzymic activities, and our laboratory is engaged in this attempt.

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