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THE INFLUENCE OF AGE, SEX AND DRUG TREATMENT ON MICROSOMAL DRUG METABOLISM IN FOUR RAT STRAINS*

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Abstract—The present study describes the influence of sex, age and drug pretreatment on drug metabolism in four common strains of rats. Basal levels of activity of hepatic microsomal drug-metabolizing enzymes in the four strains varied considerably less than those reported in different rabbit and mouse strains. There was, in general, only a 2-fold variation in metabolic rate for a single drug. Young male and female Holtzman rats showed the lowest microsomal metabolism in all drug pathways examined. Phenobarbital pretreatment caused a stimulation of drug metabolism in young and adult, male and female rats of all strains. 3-Methylcholanthrene, on the other hand, often caused no change or decreased the rate. Evidence is presented which suggests that liver microsomes may contain a number of NADPH-dependent enzymes which differ in their rates of postpubertal development.

THE ACTIVITIES of hepatic microsomal NADPH-dependent enzymes involved in the metabolism of steroids, drugs and other foreign compounds are influenced by a variety of factors. Animal species, sex, age, endocrine and nutritional status, and drug pretreatment can significantly affect these activities.[1] Thus, the activities of many microsomal drug-metabolizing enzymes are higher in mice than in dogs,[2] higher in adults than in fetal or newborn animals,[3] and higher in adult male rats than in adult females.[4] Moreover, variations in rates of drug metabolism have been found among different strains of a single animal species. Comparative studies of various strains of both mice and rabbits have been made. Jay,[5] in comparing twelve strains of mice, reported highly significant differences in hexobarbital sleeping times. These differences were subsequently confirmed by Vesell[6] and were found to correlate with differences in microsomal enzyme activity. Cram *et al.*,[7] studying six strains of rabbits, found marked differences both in basal levels of drug-metabolizing enzyme activity and in responsiveness to the enzyme inducer, phenobarbital.

The present study examines similar variations in hepatic microsomal enzyme activity among four strains of rats, the most widely employed animal species in

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studies of drug metabolism. In addition, the influence of sex, age and drug pretreatment were investigated.

METHODS

Wistar, Holtzman, Sprague–Dawley and Long–Evans rats were used in this study as representative strains of commonly used laboratory rats. Sexually mature (100 days) and immature (30 days) rats of both sexes were used. Animals were sacrificed by decapitation between 8:00 and 8:30 am and their livers were immediately excised, trimmed of extraneous material and weighed. All subsequent tissue manipulations were carried out at 0–4°. Livers were homogenized with 2 vol. of 1·15% KCl in a Polytron homogenizer (Kinematica LMBH, Luzern, Switzerland). This homogenizer gave a uniform suspension which did not differ in enzymatic activity from that produced by a Waring blendor. Homogenates were centrifuged at 9000 *g* to sediment unbroken cells, nuclei and mitochondria. The supernatant fraction was then centrifuged at 105,000 *g* for 60 min in a Spinco model L preparative ultracentrifuge and the soluble fraction was decanted. The microsomal pellet was suspended in ice-cold 1·15% KCl with the aid of a Sonifier Cell Disrupter (Heat Systems Co., Melville, N.Y.). Microsomes suspended in this manner exhibited enzyme activities comparable to those obtained by homogenization.

Reaction mixtures of 5 ml total volume were incubated for 15 min in a Dubnoff metabolic shaker at 37° with air as the gaseous phase. Each vessel contained the following constituents: NADP, 2·0 μmoles; glucose 6-phosphate, 25 μmoles; glucose 6-phosphate dehydrogenase, 1·4 units; nicotinamide, 20 μmoles; magnesium chloride, 25 μmoles; and about 10 mg of microsomal protein. Semicarbazide hydrochloride, 49 μmoles, was added to the reaction mixture when the *N*-demethylation of ethylmorphine was measured. The pH of the incubation mixture was adjusted to 7·4 with 0·1 M phosphate buffer. Preliminary studies using two of the four strains studied indicated that substrate and cofactor concentrations were above saturating levels.

The pathways studied, methods of assay and substrate concentrations were: side chain oxidation of hexobarbital,[8] 0·6 mM; *N*-demethylation of ethylmorphine,[9] 1 mM; aromatic hydroxylation of aniline,[4] mM; and *O*-demethylation of *p*-nitroanisole,[10] 2 mM.

Aliquots of the microsomal suspension were assayed for protein content by the method of Lowry *et al.*[11] with a Technicon autoanalyzer. In all cases, data are expressed as mean micromoles of substrate metabolized or product formed per milligram of microsomal protein per 15 min. Values presented are means of at least four animals. In the experiments involving phenobarbital pretreatment, the sodium salt of phenobarbital was dissolved in saline and administered (80 mg/kg, i.p.) once daily for 4 days; the last dose was given approximately 24 hr before sacrifice. Animals received 3-methylcholanthrene (40 mg/kg, i.p.) for 2 days, the last dose being given 48 hr before sacrifice.

RESULTS

Effect of drug pretreatment on microsomal protein

Phenobarbital administration resulted in significant increases in microsomal protein both in immature (30 days) and mature (100 days) animals, although the young Sprague–Dawley and Holtzman animals appeared somewhat resistant to this effect

(Table 1). 3-Methylcholanthrene treatment did not appreciably alter microsomal protein in any of the strains. It is of interest that young animals of all strains exhibited significantly lower microsomal protein levels (mg protein/g liver) than did adult animals. There were no obvious sex differences in microsomal protein content among the four strains.

Effect of drug treatment on aniline metabolism

The metabolism of aniline was significantly ($P < 0.05$) enhanced by phenobarbital pretreatment in both sexes of all strains examined (Table 2). 3-Methylcholanthrene produced no consistent change in aniline hydroxylase activity. Among the young animals, those of the Holtzman strain had the lowest basal aniline hydroxylase

TABLE 1. INFLUENCE OF DRUG PRETREATMENT ON MICROSOMAL PROTEIN LEVELS*

Strain	Age (days)	Sex	Control	Phenobarbital	3-Methylcholanthrene
Long–Evans	30	M	13·7 ± 1·3	19·3 ± 2·9	17·9 ± 1·0
	30	F	14·1 ± 1·7	19·2 ± 1·9	16·7 ± 1·7
Wistar	30	M	17·9 ± 2·1	25·8 ± 1·2	17·2 ± 2·2
	30	F	16·4 ± 0·8	27·5 ± 1·9	17·9 ± 1·1
Holtzman	30	M	20·6 ± 1·0	29·1 ± 3·3	23·4 ± 2·0
	30	F	22·0 ± 1·8	22·8 ± 8·2	19·3 ± 0·8
Sprague–Dawley	30	M	21·0 ± 1·8	23·2 ± 2·2	21·4 ± 1·5
	30	F	19·8 ± 0·9	17·2 ± 2·4	22·2 ± 2·2
Long–Evans	100	M	34·2 ± 0·9	40·0 ± 0·4	27·5 ± 1·7
	100	F	27·5 ± 1·0	36·9 ± 1·0	32·3 ± 1·4
Wistar	100	M	26·0 ± 1·0	31·6 ± 1·0	26·5 ± 0·5
	100	F	27·7 ± 0·8	27·1 ± 1·0	25·6 ± 1·7
Holtzman	100	M	28·6 ± 1·0	34·0 ± 1·0	28·3 ± 1·6
	100	F	26·0 ± 0·5	30·8 ± 2·7	24·9 ± 1·5
Sprague–Dawley	100	M	31·8 ± 0·7	42·5 ± 2·4	31·0 ± 0·9
	100	F	28·6 ± 0·7	32·9 ± 2·2	26·0 ± 1·3

* Results are expressed as mg of protein/g of liver and are presented as means ± standard error. Means are based on values obtained from at least four animals.

TABLE 2. EFFECT OF DRUG PRETREATMENT ON ANILINE METABOLISM IN FOUR RAT STRAINS*

Strain	Age (days)	Sex	Control	Phenobarbital	3-Methylcholanthrene
Long–Evans	30	M	5·2 ± 0·5	9·8 ± 1·4	5·8 ± 0·5
	30	F	5·3 ± 0·7	8·1 ± 0·7	6·5 ± 1·1
Wistar	30	M	5·2 ± 0·8	7·9 ± 0·5	7·0 ± 1·9
	30	F	6·8 ± 0·4	9·1 ± 0·2	5·7 ± 0·2
Holtzman	30	M	2·4 ± 0·4	7·9 ± 0·5	4·4 ± 0·4
	30	F	2·7 ± 0·4	7·8 ± 4·2	3·4 ± 0·3
Sprague–Dawley	30	M	4·6 ± 0·4	9·9 ± 0·7	4·1 ± 0·4
	30	F	3·0 ± 0·2	7·1 ± 0·5	3·3 ± 0·6
Long–Evans	100	M	2·2 ± 0·1	3·6 ± 0·5	3·7 ± 0·3
	100	F	1·9 ± 0·1	2·7 ± 0·2	2·2 ± 0·2
Holtzman	100	M	3·6 ± 0·2	5·7 ± 0·3	2·4 ± 0·4
	100	F	1·6 ± 0·1	2·9 ± 0·1	1·8 ± 0·3
Sprague–Dawley	100	M	2·7 ± 0·3	5·3 ± 0·3	2·5 ± 0·4
	100	F	1·7 ± 0·2	3·0 ± 0·3	1·8 ± 0·2

* Results are expressed as nanomoles of *p*-aminophenol formed/mg microsomal protein per 15 min ± standard error. The means are based on values obtained from at least four animals.

activity; this trend was not apparent in the adult animals, however. In confirmation of previous work,[12] there was no apparent sex difference in aniline metabolism in the young animals, although a difference seemed to appear in adult Holtzman and Sprague–Dawley animals (Table 2).

Effect of drug treatment on p-*nitroanisole metabolism*

Pretreatment of rats with either phenobarbital or 3-methylcholanthrene significantly ($P < 0.05$) increased the *O*-demethylation of *p*-nitroanisol by hepatic microsomes (Table 3). Induction was observed in males and females in both age groups. As

TABLE 3. EFFECT OF DRUG PRETREATMENT ON *p*-NITROANISOLE METABOLISM IN FOUR RAT STRAINS*

Strain	Age (days)	Sex	Control	Phenobarbital	3-Methylcholanthrene
Long–Evans	30	M	2·2 ± 0·4	9·4 ± 1·3	4·7 ± 1·0
	30	F	3·4 ± 0·4	14·8 ± 1·7	8·6 ± 0·8
Wistar	30	M	6·3 ± 0·7	11·9 ± 0·6	6·3 ± 0·6
	30	F	6·7 ± 0·6	12·6 ± 0·8	8·9 ± 0·6
Holtzman	30	M	1·5 ± 0·3	7·8 ± 0·7	4·9 ± 0·5
	30	F	1·9 ± 0·5	12·8 ± 1·6	5·3 ± 1·0
Sprague–Dawley	30	M	2·7 ± 0·4	11·8 ± 1·4	5·2 ± 0·3
	30	F	1·9 ± 0·3	11·5 ± 2·9	5·5 ± 0·6
Long–Evans	100	M	2·1 ± 0·3	8·1 ± 0·2	7·2 ± 0·5
	100	F	1·4 ± 0·1	5·6 ± 0·5	3·5 ± 0·3
Wistar	100	M	2·4 ± 0·2	7·4 ± 0·7	4·8 ± 0·2
	100	F	2·4 ± 0·2	8·1 ± 1·0	4·7 ± 0·2
Holtzman	100	M	2·1 ± 0·2	8·4 ± 0·2	5·2 ± 0·4
	100	F	0·6 ± 0·1	4·3 ± 0·3	2·2 ± 0·4
Sprague–Dawley	100	M	1·7 ± 0·4	7·6 ± 0·5	4·2 ± 0·2
	100	F	0·8 ± 0·1	3·8 ± 0·5	2·8 ± 0·3

*Results are expressed as nan.moles of *p*-nitrophenol formed/mg microsomal protein per 15 min ± standard error. The means are based on values from at least four animals.

was seen with aniline, the specific activities (activity/mg microsomal protein) for *p*-nitroanisole *O*-demethylation were lower in adult than in young animals, and the young Holtzman animals showed the lowest enzyme activity. No obvious sex differences were observed in young animals. Adult male rats of the Long–Evans, Holtzman and Sprague–Dawley strains metabolized *p*-nitroanisole more rapidly than did females.

Effect of drug treatment on ethylmorphine metabolism

The *N*-demethylation of ethylmorphine by hepatic microsomes was significantly ($P < 0.05$) increased in all instances by pretreatment of rats with phenobarbital, while activity was unaffected or decreased by 3-methylcholanthrene (Table 4). Marked sex differences in ethylmorphine metabolism were observed in adult animals of all four strains, males exhibiting 2–5 times as much activity as females. Furthermore, similar sex differences were noted in young animals of the Long–Evans and Sprague–Dawley strains. Again, the young Holtzman animals had the lowest enzyme activity of the four strains studied.

Effect of drug treatment on hexobarbital metabolism

Data on the microsomal metabolism of hexobarbital by four strains of rats are presented in Table 5. Phenobarbital pretreatment caused significant ($P < 0.05$)

enhancement of hexobarbital hydroxylation in male and female, young and adult animals of all strains. As was seen in the other pathways studied, the magnitude of the enhancement varied with the age, sex and strain. Administration of 3-methylcholanthrene resulted in either no change or decreased activity. As has been described by others,[13] the data show a clear sex difference in hexobarbital metabolism in all strains of adult animals. Furthermore, the 30-day-old Holtzman and Sprague–Dawley animals also showed differences in metabolism between male and female rats (Table

TABLE 4. EFFECT OF DRUG PRETREATMENT ON ETHYLMORPHINE METABOLISM IN FOUR STRAINS*

Strain	Age (days)	Sex	Control	Phenobarbital	3-Methylcholanthrene
Long–Evans	30	M	22·6 ± 0·4	66·4 ± 8·4	13·3 ± 1·9
	30	F	14·6 ± 2·1	43·3 ± 5·2	9·4 ± 0·9
Wistar	30	M	14·8 ± 1·3	31·5 ± 1·9	9·4 ± 2·7
	30	F	13·9 ± 1·0	38·6 ± 3·1	11·6 ± 2·7
Holtzman	30	M	6·2 ± 2·8	38·5 ± 3·8	10·7 ± 1·0
	30	F	4·2 ± 0·5	36·6 ± 4·5	3·5 ± 0·8
Sprague–Dawley	30	M	23·3 ± 2·5	55·3 ± 3·1	16·1 ± 1·6
	30	F	3·3 ± 0·3	24·3 ± 5·5	3·5 ± 0·6
Long–Evans	100	M	26·4 ± 4·9	45·0 ± 2·9	27·1 ± 2·8
	100	F	5·0 ± 0·4	19·8 ± 1·3	4·1 ± 0·2
Wistar	100	M	23·0 ± 4·2	37·8 ± 4·5	18·7 ± 0·9
	100	F	5·7 ± 0·5	23·7 ± 3·3	3·5 ± 0·4
Holtzman	100	M	21·6 ± 1·8	27·2 ± 1·1	16·9 ± 2·1
	100	F	8·9 ± 0·6	47·4 ± 4·5	6·8 ± 0·4
Sprague–Dawley	100	M	24·3 ± 1·4	35·7 ± 2·4	13·5 ± 1·6
	100	F	4·6 ± 0·3	20·3 ± 1·9	5·4 ± 0·5

* Results are expressed as nanomoles of formaldehyde formed/mg microsomal protein per 15 min ± standard error. The means are based on values obtained from at least four animals.

TABLE 5. EFFECT OF DRUG PRETREATMENT ON HEXOBARBITAL METABOLISM IN FOUR RAT STRAINS*

Strain	Age (days)	Sex	Control	Phenobarbital	3-Methylcholanthrene
Long–Evans	30	M	75·7 ± 10·6	142·6 ± 24·2	57·3 ± 9·5
	30	F	50·3 ± 13·2	116·7 ± 10·1	30·8 ± 4·6
Wistar	30	M	44·8 ± 12·7	89·8 ± 5·9	22·4 ± 5·1
	30	F	32·2 ± 3·0	75·4 ± 7·1	39·5 ± 5·8
Holtzman	30	M	36·3 ± 9·5	75·2 ± 9·6	27·8 ± 2·7
	30	F	6·7 ± 2·2	67·8 ± 8·8	6·0 ± 0·5
Sprague–Dawley	30	M	47·2 ± 9·9	87·5 ± 6·8	29·9 ± 6·7
	30	F	27·0 ± 2·5	122·2 ± 17·6	11·9 ± 4·1
Long–Evans	100	M	36·3 ± 3·4	85·4 ± 5·3	48·6 ± 14·1
	100	F	8·9 ± 4·6	58·2 ± 4·7	16·0 ± 2·5
Wistar	100	M	71·9 ± 11·5	77·0 ± 2·9	49·6 ± 5·2
	100	F	36·9 ± 2·7	82·4 ± 6·2	49·5 ± 3·6
Holtzman	100	M	32·1 ± 4·1	70·9 ± 4·1	43·5 ± 4·5
	100	F	17·7 ± 3·8	39·8 ± 4·5	20·5 ± 3·7
Sprague–Dawley	100	M	43·7 ± 3·3	54·0 ± 3·8	27·3 ± 4·7
	100	F	15·2 ± 3·1	51·1 ± 6·9	25·1 ± 2·7

* Results are expressed as nanomoles of hexobarbital metabolized/mg microsomal protein per 15 min ± standard error. The means are based on values obtained from at least four animals.

5). As was true for all the foregoing substrates, the lowest hexobarbital hydroxylase activity was seen in the young Holtzman animals.

DISCUSSION

Studies using mice indicate that strain variation in drug response has a genetic component which is apparent shortly after birth,[14] and the work of Law *et al.*[15] demonstrates that there may be a variation in enzymatic activity in various strains of mice. The present experiments show that the variation in basal microsomal enzyme activity in four rat strains differs by about a factor of 2. These findings are in contrast with those of Cram *et al.*,[7] who demonstrated that the variation in basal metabolic rate in rabbits was 10-fold or greater, and with those of Jay,[5] who reported a 3-fold variability in sleeping time among mouse strains, the most closely inbred strain showing the least intrastrain variation.

It is thus apparent that the wide variation in drug response and drug metabolism seen in strains of other species is not manifest in the rat. Although differences in the rate of metabolism of a single drug are seen, they are of a lesser magnitude than those observed in rabbits or mice. Among the young animals studied, both male and female Holtzman rats exhibited the lowest rates of microsomal activity in all drug pathways examined. This was not true, however, for the adult Holtzman animals. Their enzymatic activity was of approximately the same magnitude as that of the other three strains.

Among the untreated animals, with the exception of the Holtzman strain, it is noteworthy that specific activities (activity/mg microsomal protein) were consistently higher in young animals than in adults. This may represent a difference in the kinetic properties of the enzymes, e.g. turnover number, or may simply reflect an accumulation of enzymatically inert protein in microsomes of adult animals.

Although it is clear that a sex difference for the hepatic microsomal metabolism of several drugs develops in association with puberty,[16] data in the present paper suggest that maturation of drug-metabolizing capacity does not occur for all enzymes simultaneously. For example, in the 30-day-old animals, there were no statistically significant sex differences in the metabolism of *p*-nitroanisole, even though a significant sex difference in the metabolism of this substrate was observed in adult animals. In the same group of 30-day-old animals, however, there was a significant sex difference for the metabolism of ethylmorphine by the Long–Evans and Sprague–Dawley strains and for the metabolism of hexobarbital by the Holtzman and Sprague–Dawley strains. These findings suggest that hepatic microsomes contain a family of NADPH-dependent enzymes which may differ in their rates of postpubertal development. Similar findings have been published demonstrating differential rates of postnatal development[17] among microsomal enzymes. Therefore, in accord with the conclusions of others,[18] the present data should be considered before the theory of a single microsomal "oxidase" can be accepted.

There were no conspicuous instances of age, sex or strain combinations in which refractoriness to enzyme inducers was observed. In general, when phenobarbital or 3-methylcholanthrene cause stimulation of the metabolism of a given substrate, stimulation was observed in all strains, in both sexes, and in young and adult animals. Phenobarbital administration was found to either maintain a sex difference in drug metabolism, to abolish it or to reverse it. It is to be emphasized, however, that the

magnitude of the stimulation varied with the age, sex and strain under consideration.

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