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MICROSOMAL EPOXIDE HYDROLASE IN DIFFERENT RAT STRAINS

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Abstract—Epoxide hydrolase activity was determined in hepatic microsomes of adult males of 22 rat strains. The specific activity varied between 4.3 and 12.7 nmole styrene glycol/mg protein per min. The enzyme in F344, DA and Sprague–Dawley rats, strains with low, high and intermediate activity, respectively, was studied in more detail. No differences in substrate specificity and pH-dependence of the activity were observed between the strains with high and low activity, and immunoprecipitation by antibodies raised against microsomal epoxide hydrolase purified from Sprague–Dawley rats showed that the amounts of enzyme protein in microsomes from DA and F344 rats correlated with the activities. These results indicate quantitative rather than qualitative differences in epoxide hydrolase. The enzyme activity was inherited in an autosomal and codominant manner. The hepatic activity in females (about 78% of that in males) and, with the limitation that only few situations were studied, the *trans*-stilbene oxide-induced activity were under the same genetic control as the basal hepatic activity in males. In contrast, some extrahepatic tissues showed strain differences in epoxide hydrolase activity which contrasted with those found in liver. Hence, the enzyme activity in one tissue cannot serve as a reliable guide to the relative activity in another tissue, unless a specific correlation between the two tissues has been established. Although the strain differences in activity were not very large in themselves, in combination with inter-individual variation, sex differences and effects of the enzyme inducer *trans*-stilbene oxide they led to a 20-fold variation in hepatic epoxide hydrolase activity among the rats investigated in the present study.

Aromatic and olefinic compounds can be metabolized by mammalian monooxygenases to electrophilically reactive epoxides, which may covalently bind to cell components and thereby cause mutations, initiation of tumours or other toxic effects [1, 2]. Microsomal epoxide hydrolase (epoxide hydratase, epoxide hydrase, EC 3.3.2.3) inactivates many epoxides by conversion to *trans*-dihydrodiols [2–4]. Inasmuch as some dihydrodiols derived from polycyclic aromatic hydrocarbons, are further oxidized by monooxygenases to highly reactive vicinal dihydrodiol-epoxides [5, 6], epoxide hydrolase is sometimes also involved in metabolic activation. It can be expected that different levels of epoxide hydrolase activity will affect the toxicity of many compounds.

Microsomal epoxide hydrolase activity has been observed in all investigated vertebrate species [7] and in numerous organs and tissues of the rat [8]. However, the differences in activity among species and organs were very large [7–9]. In a study on 166 human individuals, mostly suffering from liver diseases, a 63-fold inter-individual variation in epoxide hydrolase activity in liver microsomes was observed [10]. Mice showed a genetic polymorphism of the enzyme with respect to the pH optimum [11, 12]. However, as this polymorphism led to large differences in enzyme activity only at pHs above 9.0, it is probably of low biological significance. At pH 9.0 and below, maximal differences in activity between various mouse strains were only about 2-fold [7, 11–13]. The present study was therefore performed on rats to investigate whether they provide a better model than mice for human inter-individual variation, and also because of their wide use in toxicological studies.

MATERIALS AND METHODS

Chemicals. [7-^{3}H]styrene 7,8-oxide (75 mCi/mole), [G-^{3}H]benzo[*a*]pyrene 4,5-oxide (432 mCi/mole) and [17-^{3}H]16α,17α-epoxy-1,3,5(10)-estratrien-3-ol (estroxide, 3.1 Ci/mole) were synthesized as published [14–16].

Animals. The different rat strains were obtained from F. Hoffman–La Roche & Co. (Basel, Switzerland), Zentralinstitut für Versuchstiere (Hannover, F.R.G.), Olac 1976 Ltd. (Bicester, U.K.) and Versuchstier-Zuchtanstalt WIGA (Sulzfeld, F.R.G.), as indicated in Table 1. DA and F344 rats used in later experiments were always from Zentralinstitut für Versuchstiere (Hannover) and Sprague–Dawley rats from WIGA (Sulzfeld). Before use in experiments, the animals were kept for at least 7 days under uniform conditions, i.e. a day–night cycle of 12:12 hr, a temperature of 21–24°, and free access to water and a defined diet (Altromin). All animals used in experiments had body weights of 170–250 g.

Enzyme assay. Microsomal fractions were prepared as described previously [8]. Protein concen-

Table 1. Epoxide hydrolase activity in liver microsomes of different rat strains*

Strain	Mode of breeding	Source	Specific activity (nmole styrene glycol/mg protein per min)
F344/Ola (Fischer)	Inbred	Bicester	4.3 ± 0.7
PVG/Ola	Inbred	Bicester	4.6 ± 0.5
Fischer 344	Inbred	Basel	4.7 ± 0.2
ANG/Ola	Inbred	Bicester	4.9 ± 1.0
F344/NHan (Fischer)	Inbred	Hannover	5.2 ± 0.8
LE/Han (Long–Evans)	Inbred	Hannover	6.3 ± 1.0
Han: Wistar	Outbred	Hannover	6.5 ± 0.7
Sprague–Dawley	Outbred	Sulzfeld	7.1 ± 1.9
WAG/Ola	Inbred	Bicester	7.2 ± 1.2
LEW/Han (Lewis)	Inbred	Hannover	7.2 ± 1.8
Han: Sprague–Dawley	Outbred	Hannover	7.6 ± 1.6
E3/Han	Inbred	Hannover	7.9 ± 0.5
Fawn-hooded	Inbred	Basel	8.1 ± 0.9
Lewis	Inbred	Basel	8.1 ± 2.5
BDE/Han	Inbred	Hannover	8.2 ± 0.4
Piebald	Inbred	Basel	9.0 ± 3.0
SHR/Jap Nu	Inbred	Basel	9.1 ± 1.1
Fü-Albino	Inbred	Basel	9.1 ± 4.4
DA × Lewis	F_1-Hybrid	Basel	9.5 ± 3.1
Holtzman	Inbred	Basel	10.6 ± 3.2
DA/Bas	Inbred	Basel	12.5 ± 4.3
DA/Han	Inbred	Hannover	12.7 ± 2.4

* Male rats were obtained from Hoffmann–La Roche (Basel, Switzerland), Zentralinstitut für Versuchstiere (Hannover, F.R.G.), Olac (Bicester, U.K.) and Versuchstier-Zuchtanstalt Wiga (Sulzfeld, F.R.G.) and kept under uniform conditions for 7–14 days, before epoxide hydrolase activity (with styrene 7,8-oxide as the substrate) was determined. The body weight of all animals was between 180 and 220 g. Values are means and S.D. of determinations of four animals.

trations were determined by the method of Lowry *et al.* [17] with bovine serum albumin as the standard. Epoxide hydrolase activity was estimated using the radiometric extraction assays with styrene 7,8-oxide [14], benzo[*a*]pyrene 4,5-oxide [18] and estroxide [19] as substrates. The assay with styrene 7,8-oxide was performed without addition of Tween 80 [20].

RESULTS

Strain differences in liver epoxide hydrolase

The specific activities of epoxide hydrolase (with styrene 7,8-oxide as the substrate) in liver microsomes from adult male rats of 22 investigated strains ranged from 4.3 to 12.7 nmole/mg protein per min (Table 1). F344, Sprague–Dawley, Lewis or DA rats from different breeding farms differed in epoxide hydrolase activity only minimally and the differences were not statistically significant.

Two strains with widely different activities, F344 (Hannover) and DA (Hannover), were selected for a comparative study of some properties of the enzyme. Figure 1 shows that the pH-dependence of the epoxide hydrolase activity was very similar in the two strains. As in Sprague–Dawley rats [14], the optimal pH was at 9.0–9.5 with a steep decrease in activity at higher pH and a very slow decrease at lower pH.

Styrene 7,8-oxide is a relatively simple alkene oxide. Benzo[*a*]pyrene 4,5-oxide is an arene oxide. Both are xenobiotics, whereas the steroid estroxide is an endogenous substrate [19] of epoxide hydrolase. Towards these different epoxides the specific activity of liver microsomes from DA rats was uniformly 2.5- to 2.8-fold greater than that of F344 rats (Table

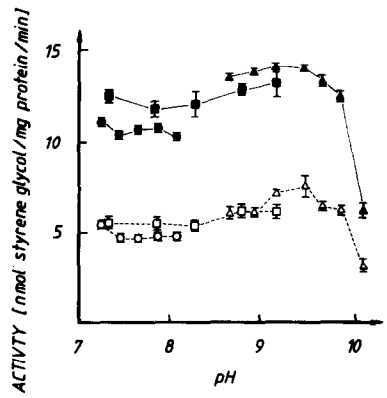

Fig. 1. pH-Dependence of epoxide hydrolase activity in hepatic microsomes from F344 (○, □, △) and DA (●, ■, ▲) rats. Microsomes were prepared from adult male animals. Epoxide hydrolase activity was determined as described in Materials and Methods except that the following buffers (at a final concentration of 100 mM) were used: sodium phosphate (○, ●); Tris–HCl (□, ■); glycine–NaOH (△, ▲). The pH was determined directly in the incubation mixture. All values were obtained under conditions where activity was linear with the amount of protein. Points are means ± S.D. of four replicate determinations.

Table 2. Substrate specificity of microsomal epoxide hydrolase from DA and F344 rats*

Rat strain	Specific activity (nmole diol/mg protein per min)		
	Styrene 7,8-oxide	Benzo[*a*]pyrene 4,5-oxide	Estroxide
DA	8.80 ± 0.02	6.05 ± 0.12	10.5 ± 0.40
F344	3.18 ± 0.00	2.44 ± 0.11	4.17 ± 0.37
Ratio DA/F344	2.8	2.5	2.5

* Liver microsomes were prepared from adult male DA and F344 rats. Values are means ± S.D. from quadruplicate determinations of the enzyme activities.

2), indicating similar substrate specificities of the epoxide hydrolase in the two strains. The relative activities towards the three substrates also coincide with those in Sprague–Dawley liver microsomes [8, 19].

Antibodies raised against homogeneous Sprague–Dawley epoxide hydrolase precipitated epoxide hydrolase in solubilized hepatic microsomes from both F344 and DA rats (Fig. 2). At high concentrations of antibodies, precipitation was complete in both cases. About three times more antiserum was required for precipitation of the same proportion of enzyme from DA microsomes as from an identical amount of F344 microsomes. This agrees with the three times greater specific activity in the former strain.

Mode of inheritance of hepatic epoxide hydrolase

F_1 hybrids of F344 and Sprague–Dawley rats had epoxide hydrolase activities which were intermediate to those of the parent strains (Table 3). This was the case for both the male and female progeny and was independent of whether the mother or the father was of the F344 strain. The same autosomal additive mode of inheritance was found in intercrosses of F344 and DA rats (Table 4). Also, the male F_1 progeny of female DA and male Lewis parents had intermediate epoxide hydrolase activities (Table 1) (the inverse crossing and the female progeny were not studied).

A tentative backcrossing of F_1 hybrids of F344 and Sprague–Dawley rats with the parent strains (data not shown) led to mean epoxide hydrolase activities in the progeny which were between the activities of the F_1 and the homozygous strain, but with relatively large inter-individual variations. The results were well compatible with a 1:1 segregation of the backcross generation in animals with the activity of the F_1 and of the homozygous strain, which would be expected with a monogenic mode of inheritance. However, because of the overlapping of the activities in backcrosses, F_1 and parent strain, alternative genetic controls cannot be excluded.

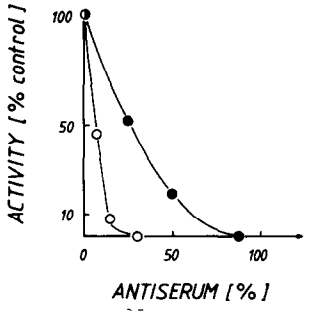

Fig. 2. Immunoprecipitation of epoxide hydrolase activity from solubilized hepatic microsomes of F344 (○) and DA (●) rats. Microsomal suspensions with a protein content of 8 mg/ml were prepared from adult male rats and solubilized by addition of sodium cholate (final concentration 1%). Antibodies to purified epoxide hydrolase were raised in New Zealand White rabbits [22]. Various amounts of antiserum were added to the solubilized microsomes. In order that all incubations contained the same total amount of serum, they were complemented with control serum. After incubation at 0–4° for 18 hr, antigen–antibody complexes were sedimented by centrifugation at 45,000 *g* for 10 min and the supernatant fractions were assayed for epoxide hydrolase activity with styrene 7,8-oxide as the substrate. The microsomal preparations used had specific activities of 3.8 (F344 rats) and 11.9 (DA rats) nmole styrene glycol/mg protein per min. The initial slopes of precipitation were 0.54 (F344 rats) and 0.48 (DA rats) nmole styrene glycol/min per μl antiserum.

Epoxide hydrolase in the liver of female rats

In addition to other results, the data in Tables 3 and 4 show that in all investigated cases females possessed lower epoxide hydrolase activity than males (71–88%) and strain differences in activity paralleled those in males.

Induction of liver epoxide hydrolase activity in F344 and DA rats

Epoxide hydrolase activity in both F344 and DA rats was induced by *trans*-stilbene oxide (Table 5) to a similar extent to Sprague–Dawley rats [21]. The increases in activity were approximately proportional to the basal activities, i.e. the absolute increases were larger in rats with a high basal activity than in those with a low basal activity.

Epoxide hydrolase in extrahepatic tissues

Extrahepatic tissues of the rat possess lower epoxide hydrolase than the liver [8]. Therefore, benzo[*a*]pyrene 4,5-oxide, which allows detection of lower enzyme activities than styrene 7,8-oxide [18], was used as the substrate in experiments where epoxide hydrolase activity in various organs was compared in F344, Sprague–Dawley and DA rats. The variation of the activities among the three strains was different in different organs (Fig. 3). As an example, DA rats had statistically significantly higher activities in liver and skin (cutis), but lower activities in testis and kidney than F344 rats, whereas the

Table 3. Epoxide hydrolase activity in liver microsomes from F344 and Sprague–Dawley rats and their F_1 intercrosses*

	Epoxide hydrolase activity		
		Females	
Intercross (mother × father)	Males (nmole styrene glycol/mg protein per min)	(nmole styrene glycol/mg protein per min)	Compared with males (%)
F344 × F344	3.63 ± 0.35 (3)	2.73 ± 0.17 (4)	75
F344 × Sprague–Dawley	4.88 ± 1.03 (8)	3.79 ± 0.80 (7)	78
Sprague–Dawley × F344	4.86 ± 1.08 (10)	4.28 ± 0.99 (4)	88
Sprague–Dawley × Sprague–Dawley	6.58 ± 0.71 (10)	4.66 ± 0.98 (8)	71

* The different intercrosses were raised in our laboratory at the same time and under uniform conditions. Epoxide hydrolase was determined when the animals had reached a body weight of 180–250 g, i.e. at the age of about 2 months for the males and 4 months for the females (i.e. both in young adults, both having similar body weights). Values are means ± S.D. from determinations in individual animals. The numbers of individuals are given in parentheses.

Table 4. Epoxide hydrolase activity in liver microsomes from F344 and DA rats and their F_1 intercrosses*

	Epoxide hydrolase activity		
		Females	
Intercross (mother × father)	Males (nmole styrene glycol/mg protein per min)	(nmole styrene glycol/mg protein per min)	Compared with males (%)
F344 × F344	5.61 ± 0.16 (2)	4.42 ± 0.57 (4)	79
F344 × DA	7.61 ± 1.48 (8)	5.94 ± 0.98 (13)	78
DA × F344	7.45 ± 1.84 (6)	5.80 ± 0.16 (2)	78
DA × DA	9.39 ± 0.01 (2)	6.86 ± 0.95 (4)	73

* See Table 3.

Table 5. Induction of liver epoxide hydrolase activity by *trans*-stilbene oxide in F344 and DA rats*

	Specific activity (nmole styrene glycol/mg protein per min)			
Strain	Control rats	*trans*-Stilbene oxide-treated rats	Difference	Fold increase in activity
F344	3.6 ± 0.7	18.0 ± 5.2	14.4	5.0
DA	8.4 ± 0.5	34.3 ± 4.7	25.9	4.1

* Adult males received three intraperitoneal injections of 400 mg *trans*-stilbene 7,8-oxide (in 1 ml sunflower oil) per kg body weight or only sunflower oil at 24 hr intervals. They were killed 24 hr after the last treatment. Values are means ± S.D. of determinations in four animals.

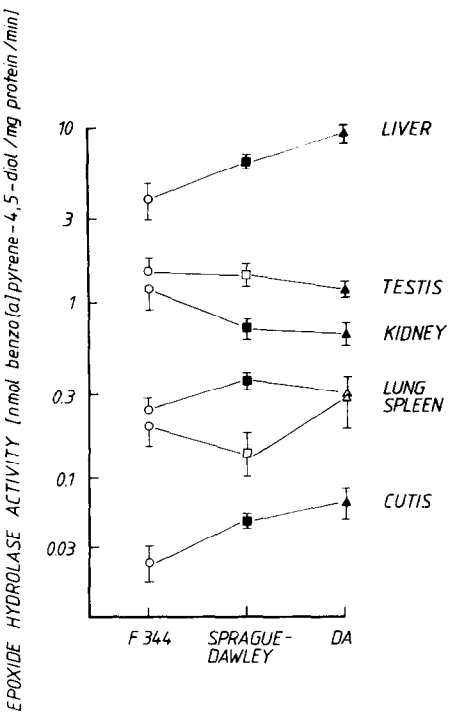

Fig. 3. Strain differences in epoxide hydrolase activity in various organs of the rat. Epoxide hydrolase activity, with benzo[*a*]pyrene 4,5-oxide as the substrate, was determined in microsomes from the pooled organs of 3–5 adult rats. All determinations were controlled for linearity with protein. Values are means ± S.D. of the activities in microsomal preparations from three independent pools of organs, except for the Sprague–Dawley rat where previously published data [8] are shown. Full symbols indicate that the results were significantly different ($P < 0.05$, *t*-test) from those with F344 rats.

activities in lung and spleen did not significantly differ in the two strains.

DISCUSSION

Differences in epoxide hydrolase activity in hepatic microsomes were found in different rat strains. The strains with the lowest and highest activities were F344 and DA rats. The variation among F344 or DA rats obtained from different sources or raised in our laboratory at different times was always smaller than the 2- to 3-fold differences which occurred between F344 and DA rats. This indicated a genetic rather than an environmental cause.

Interestingly, F344 and DA rats are also prototypes of another polymorphism in drug metabolism: hepatic microsomes from DA rats form much less 4-hydroxydebrisoquine from debrisoquine than do microsomes from F344 rats [23]. This correlation with differences in epoxide hydrolase activity most likely is fortuitous, as no connection is recognizable in other strains (Wistar, Lewis) in which both debrisoquine metabolism [24] and epoxide hydrolase (this study) have been investigated.

Epoxide hydrolase in F344 and DA rats did not differ with respect to substrate specificity, dependence of the activity on pH, or immunological properties. Also, these properties correspond well with data in the literature on Sprague–Dawley epoxide hydrolase. This suggests that quantitative rather than qualitative differences in the enzyme were responsible for the strain differences in activity and contrasts with the situation in mice, which can be separated into two groups of strains with different pH-optima for the epoxide hydrolase activity [11, 12]. As in the mouse [12, 13], rat hepatic epoxide hydrolase activity was inherited in an autosomal, additive manner.

Females possessed somewhat lower hepatic epoxide hydrolase activities than males, but strain differences were parallel in both sexes, indicating a common genetic control. With the limitation that only few situations were investigated, this may also apply to the *trans*-stilbene oxide-induced activity, as indicated by the multiplicative (rather than additive) character of induction. In contrast, some extrahepatic tissues showed completely different strain differences in (basal) epoxide hydrolase activity. Hence, measurement of enzyme activity in one tissue cannot be used to predict the activity in another tissue, unless a specific correlation between the two tissues has been proved.

Epoxide hydrolase activities in various tissues varied at most by about 3-fold among different strains. An approximately 2-fold interstrain variation was previously reported for the basal activity in mouse liver [7, 12, 13]. These interstrain variations are small in comparison to interspecies variations. Also different species, in contrast to rat strains, greatly differ in the organ distribution of epoxide hydrolase [8, 9]. In man, a 63-fold inter-individual variation of hepatic epoxide hydrolase activity was observed [10]. However, most of the subjects studied suffered from liver diseases and were treated with drugs, whereas in this study we used only healthy adult rats which had similar body weights, were kept under uniform conditions and were not treated with enzyme inducers except the animals which were induced with *trans*-stilbene oxide. The lowest observed hepatic epoxide hydrolase activity was 2.0 nmole styrene glycol/mg protein per min (in an untreated F344 female), which was 20-fold lower than the highest activity, 40.5 nmole styrene glycol/mg protein per min (in a *trans*-stilbene oxide-induced DA male). It can be expected that under more extreme physiological conditions the variability in hepatic epoxide hydrolase activity in rats could be as much as that observed in man. However, this is not normally the case in toxicological studies in animals. The difference in variation should be kept in mind when extrapolating to man where individuals with extremely high or low activities occur, as epoxide hydrolase activity very likely may affect the toxicity of various compounds.

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