Biochemical Pharmacology, Vol. 20, pp. 2695–2701. Pergamon Press, 1971. Printed in Great Britain

RAT STRAIN DIFFERENCES IN THE ACTIVITY OF HEPATIC MICROSOMAL ENZYMES

A. JORI, R. PESCADOR and C. PUGLIATTI

Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea, 62–20157 Milano, Italy

(*Received* 18 *February* 1971; *accepted* 7 *April* 1971)

Abstract—Drug metabolism in Sprague–Dawley and Long–Evans rats, from two different sources (A and B), was studied *in vitro* and *in vivo*. Female Long–Evans (A) rats, but not Long–Evans (B) rats, metabolize pNO_2 anisol, aminopyrine, hexobarbital and imipramine *in vitro* to a lower extent than Sprague–Dawley rats. Marked sex differences in this respect are evident in both strains.

In vivo a reduced elimination of aminopyrine from plasma was obtained in Long–Evans rats (A) as compared to animals from the (B) source. Moreover tissue concentration of imipramine, aminopyrine and phenobarbital were higher Long–Evans (A) than in Sprague–Dawley. The disappearance from plasma of amphetamine and zoxazolamine was apparently not different in the two strains.

STRAIN differences in the activity of the hepatic microsomal enzymes responsible for drug metabolism have been reported for several animal species.

Variations in pharmacological response to barbiturates were observed in mice[1–3] and correlated to differences in microsomal enzyme activity.[4] Differences in drug metabolizing enzyme activity of six strains of rabbits were reported by Cram *et al.*[5] both in basal conditions and after induction. Minor differences were found in microsomal enzyme activity among rat strains.[6–9]

In previous studies[10] on the metabolism of imipramine in rats, we have found no differences *in vitro* and *in vivo* among some of the most commonly used strains of rat, such as Wistar, Sprague–Dawley and Holtzmann. On the contrary Long–Evans rats showed a reduced metabolic activity particularly when *N*-demethylation reactions were investigated, as in the case of the metabolism of imipramine and aminopyrine.

The aim of the present study is to examine the differences in drug metabolism between Sprague–Dawley and Long–Evans rats of both sexes and from two sources.

MATERIALS AND METHODS

Female and male Sprague–Dawley (ALAL, Como, Italy) and Long–Evans rats (A) Les Laboratoires Servier, Orléans, France and (B) Allevamento Morini (San Polo d'Enza, Reggio Emilia, Italy) weighing 180–220 g were used. All the animals were housed for at least 10 days in our animal rooms (temperature 22° and 56 per cent of relative humidity) and fed a balanced standard diet (ALAL Co., Italy) and water *ad lib.* before the experiment.

In vitro *experiments*

The rats were killed by decapitation and the livers were rapidly removed, placed in dry ice and stored at −20°. Immediately before the determinations of the enzymatic

activity, the stored livers were homogenized in 1·15% KCl solution. This homogenate was centrifuged (9000 *g*) at 4° and the supernatant fraction was used.

The incubation mixtures consisted of 9000 *g* supernatant equivalent to 640 mg of liver, 50 μmoles of glucose-6-phosphate, 0·70 U.I. glucose-6-phosphate dehydrogenase (only for microsomes) 1·5 μmoles of NADP, 100 μmoles of nicotinamide, 25 μmoles of $MgCl_2$, 1·4 ml of 0·2 M sodium phosphate buffer, the substrate to be metabolized up to a final volume of 5 ml.

The following concentrations of substrates were used: pNO_2anisol 1·5 μM; aniline 5 μM; aminopyrine 5 μM; hexobarbital 4 μM; imipramine 1 μM.

The incubation was performed at 37° in air, for 30 min in a shaker (140 oscillations/min).

The hydroxylation of hexobarbital was measured by following the disappearance of the substrate according to the method of Cooper and Brodie;[11] the hydroxylation of aniline was determined by measuring the formation of pNH_2 phenol according to Gilbert and Golberg;[12] the *N*-demethylation of aminopyrine was measured by following the appearance of 4-aminoantipyrine according to La Du;[13] the *O*-demethylation of p-NO_2anisol was determined by the formation of pNO_2phenol.[12] Determinations of hepatic proteins in 9000 *g* fraction were performed according to the method of Lowry *et al.*[14]

In vivo *experiments*

Female rats were given aminopyrine (40 mg/kg orally or 16 mg/kg i.v.), *d*-amphetamine-sulphate (15 mg/kg i.p.), zoxazolamine (50 mg/kg i.v.), imipramine (20 mg/kg i.p.) and phenobarbital (50 mg/kg i.p. daily for 3 days). The concentrations in plasma and/or in brain were determined at various times, as it is reported in details under the tables.

The following methods of determinations were used: for aminopyrine[15] for amphetamine;[16] for zoxazolamine;[17] for imipramine and desipramine;[18] for phenobarbital.[19]

TABLE 1. PLASMA AND BRAIN CONCENTRATIONS OF VARIOUS DRUGS IN LONG–EVANS (A) AND SPRAGUE–DAWLEY RATS

No. of rats	Treatment (mg/kg)	Sprague–Dawley Drug concentration		Long–Evans (A) Drug concentration	
		Plasma (μg/ml ± S.E.)	Brain (μg/g ± S.E.)	Plasma (μg/ml ± S.E.)	Brain (μg/g ± S.E.)
6	Aminopyrine 40, oral	5·0 ± 0·8	—	14·2 ± 1·1*	—
8	Imipramine 20, i.p.	—	11 ± 0·9	—	20·3 ± 2·1*
5	Phenobarbital 50, i.p.	48·7 ± 3·3	54 ± 2	78 ± 2*	92 ± 3*
6	Amphetamine 15, i.p.	1·2 ± 0·2	9·6 ± 1	1·4 ± 0·2	8·9 ± 1·1
6	Zoxazolamine 50 i.v.	7·0 ± 0·8	12·5 ± 2·6	8·2 ± 0·8	13·3 ± 3·6

Female rats weighing about 200 g ± 5 were used. Determinations were performed 6 hr after aminopyrine, 90 min after imipramine, 3 hr after *d*-amphetamine sulphate, 8 hr after phenobarbital (given daily for 3 days) and 4 hr after zoxazolamine.

* $P < 0·01$ versus Sprague–Dawley rats.

RESULTS

Table 1 reports the concentrations in plasma and/or in brain of various drugs after a treatment in Long–Evans (A) and Sprague–Dawley rats. It may be noted that the levels of aminopyrine, imipramine and phenobarbital are significantly higher in Long–Evans than in Sprague–Dawley rats. Drugs which are mostly hydroxylated in the aromatic ring such as amphetamine and zoxazolamine do not show differences according to the strain of rats utilized. Figure 1 shows the disappearance of orally given aminopyrine in the two strains of rats.

When the plasma concentrations are plotted against the time in a semilogarithmic paper the rate of disappearance of aminopyrine in Long–Evans (A) is significantly slower than in Sprague–Dawley rats.

After intravenous injection of aminopyrine in female rats there is a similar rate of disappearance of the drug from the blood stream of Long–Evans (B) and Sprague–Dawley but a lower rate of disappearance for Long–Evans (A) (Fig. 2).

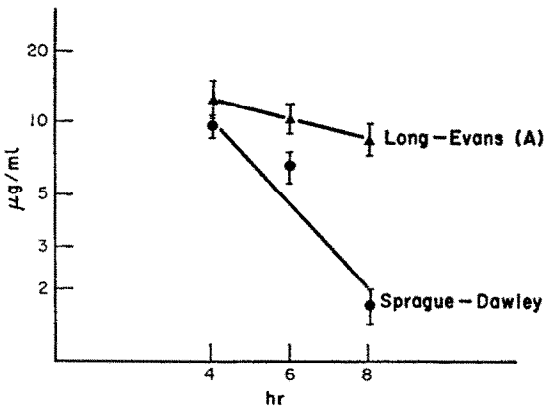

FIG. 1. Levels of aminopyrine in plasma (μg/ml) of Long–Evans (A) (▲) and Sprague–Dawley (●) rats after an oral administration of aminopyrine (40 mg/kg). The vertical bars represent the standard error of the mean.

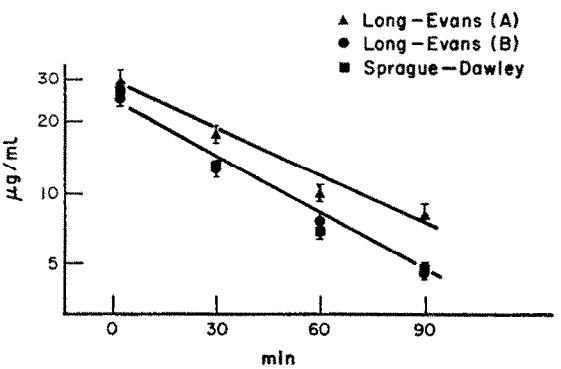

FIG. 2. Levels of aminopyrine in plasma (μg/ml) of Long–Evans (A) (▲); (B) (●) and Sprague–Dawley (■) rats after an intravenous administration of aminopyrine (16 mg/kg). The vertical bars represent the standard error of the mean. The two regression lines are not parallel. The "slopes" (b) are significantly different ($P < 0.05$) with the *t*-test.

TABLE 2. *In vitro* METABOLISM OF VARIOUS SUBSTRATES BY 9000 *g* SUPERNATANT OF LIVER, MALE AND FEMALE, SPRAGUE–DAWLEY AND LONG–EVANS RATS

Strain	Sex	Body weight (g ± S.E.)	Liver weight (g ± S.E.)	Proteins 9000 *g* sup. (mg/g liver)	pNO_2 phenol	pNH_2 phenol	$4NH_2$ antipyrine	Hexobarbital	Imipramine	DMI
						(mμmoles/g liver/hr ± S.E.)				
Sprague–Dawley	♂	225 ± 3	10·1 ± 0·3	153 ± 6	774 ± 61	710 ± 69	406 ± 45	4178 ± 476	2486 ± 161	1172 ± 244
Long–Evans (A)	♂	239 ± 6	9·2 ± 0·2*	154 ± 6	897 ± 35	799 ± 23	538 ± 15*	8432 ± 323†	2929 ± 24*	1852 ± 65*
Sprague–Dawley	♀	221 ± 2	8·6 ± 0·3	158 ± 2	533 ± 55	417 ± 47	150 ± 16	1645 ± 140	2030 ± 89	944 ± 71
Long–Evans (A)	♀	215 ± 2	7·2 ± 0·3†	150 ± 7	378 ± 94*	467 ± 48	88 ± 16*	839 ± 99†	1503 ± 66†	604 ± 31†

Long–Evans rats (A) supplied by Les Laboratoires Servier.
DMI and imipramine were measured in the same sample.
Each figure is the mean of four experiments. The figures represent *p*-nitrophenol formed from *p*-nitroanisol, *p*-aminophenol formed from aniline, 4 amino-antipyrine formed from aminopyrine, hexobarbital disappeared, desipramine formed from the corresponding imipramine disappeared.
* $P < 0{\cdot}05$ versus the group of Sprague–Dawley rats of the same sex.
† $P < 0{\cdot}01$.

In Table 2 the *in vitro* liver microsomal enzymatic activity of Long–Evans and Sprague–Dawley rats are compared in respect of the sex.

Female Long–Evans rats metabolize to a minor extent pNO_2anisol, aminopyrine and hexobarbital as compared to Sprague–Dawley females. Formation of desipramine from imipramine is also reduced in female Long–Evans rats. No significant differences are observed for the aromatic hydroxylation of aniline.

On the contrary male Long–Evans rats show an increased formation of desipramine from imipramine, of 4-amino antipyrine from aminopyrine and an increased disappearance of hexobarbital in respect to the male Sprague–Dawley rats.

Table 3 reports the enzymatic activity for the aminopyrine demethylation obtained by female Sprague–Dawley rats and female Long–Evans rats from two different sources. One of these (A) shows the usual low metabolic activity in liver microsomal preparations. On the contrary Long–Evans rats from source B do not show any significant difference in respect to the Sprague–Dawley rats at least for the parameters considered, except a lower protein concentration in 9000 *g* supernatant fraction.

TABLE 3. *In vitro* METABOLISM OF AMINOPYRINE FROM LIVER PREPARATIONS OF FEMALE SPRAGUE–DAWLEY AND LONG–EVANS RATS

Strain	Rat weight (g ± S.E.)	Liver weight (g ± S.E.)	Proteins 9000 *g* supernatant (mg/g liver ± S.E.)	$4NH_2$ Antipyrine* 9000 *g* superanatant fraction (mμmoles/g liver/hr ± S.E.)	$4NH_2$ Antipyrine* 9000 *g* superanatant fraction (mμmoles/total liver/hr ± S.E.)
Sprague–Dawley	210 ± 2	8 ± 0·15	162·1 ± 6	208 ± 11	1756 ± 90
Long–Evans (A)	206 ± 1	7 ± 0·16†	159 ± 7	107 ± 11‡	721 ± 69‡
Long–Evans (B)	197 ± 3	8 ± 0·16	116·8 ± 4§	201 ± 25	1608 ± 197

Long–Evans rats (A) supplied by Les Laboratoires Servier.
Long–Evans rats (B) supplied by Allevamento Morini.
* The figures represent the formation of 4 aminoantipyrine from aminopyrine.
† $P < 0·001$.
‡ $P < 0·05$.
§ $P < 0·02$: versus Sprague–Dawley rats.

Data not reported here in detail show that no differences with respect to apparent K_m (K_m [M] $0·526 \times 10^{-3}$) exist among strains and stocks considered in this study for the *N*-demethylation of aminopyrine but approximately 2·5-fold differences in respect to V_{max} are present between the Long–Evans of the two sources. The weight of the liver in Long–Evans rats (A) was found to be significantly lower than in the other groups of rats (Tables 2 and 3) and consequently also the whole liver capacity of metabolizing aminopyrine is lower in these rats than should be expected from the data reporting the substrate metabolized from 1 g of fresh tissue (see Table 3).

DISCUSSION

The data here reported confirm our previous results and indicate that female Long–Evans rats (A) metabolize some drugs to a lesser extent as compared to the corres-

ponding Sprague–Dawley animals. These findings are supported by the results obtained by measuring *in vitro* the enzyme activity on the 9000 *g* fraction and *in vivo* by the rate of disappearance from the plasma or the tissue levels of several drugs.

In addition to the different specific enzyme activity of liver microsomes, it may be noted that another important factor responsible for the reduced metabolism of drugs *in vivo* in Long–Evans (A) rats, is represented by the size of liver. In fact the weight of the liver of the 180 rats examined expressed as gram per cent body weight is 3·95 ± 0·05 in Sprague–Dawley but it is only 3·11 ± 0·04 in Long–Evans (A) rats.

A marked sex difference on the *in vitro* metabolism is present in both the strains according to the data reported by others[8,20,21]. The male Long–Evans rats show a higher enzyme activity than the male Sprague–Dawley animals for the metabolism of some substrates *in vitro*.

However, our results stress another interesting point concerning differences between rats of the same strain.

In fact quite different results are obtained when Long–Evans rats from other sources (B) are employed in the same environmental and experimental conditions.

Long–Evans (B) show in fact no variations in drug metabolism in respect to Sprague–Dawley and therefore they exhibit differences when compared to Long–Evans rats (A).

These results are not surprising because such discrepancies are reported by other authors.

The liver weight (gram per cent body wt.) in Long–Evans rats was found to be 4·2 by Freudenberger[22] and 2·7 by Kozma.[23]

As the metabolic activity concerns, Furner[7,8] observed not more than 2-fold difference in basal microsomal activity in four adult rat strains including Long–Evans. On the other hand Quinn[2] demonstrated pronounced variations in the biological half-life of antipyrine in eight inbred strains of rats.

Page and Vesell[9] in ten different strains of Rattus Norvegicus found differences up to 88 per cent in the V_{max} values for the metabolism of aniline and ethylmorphine.

It should be emphasized therefore that the information obtained by Long–Evans rats and possibly other strains should not be interpreted as applicable to all Long–Evans stocks.

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