THE EFFECT OF CASTRATION AND TESTOSTERONE ON SOME COMPONENTS OF THE MICROSOMAL DRUG METABOLISING ENZYME SYSTEM IN MICE

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1. Introduction

Sex differences in the rate of drug metabolism have been described for rats [1, 2]. Male rats metabolise some drugs faster than females. In mice some authors failed to demonstrate a sex difference in drug metabolism [1, 3, 4], while others found that the metabolism of hexobarbital [5] and the demethylation of ethylmorphine [6] take place faster in female than in male mice. These apparent discrepancies were explained by Vesell [7], who found that the sex difference in the rate of hexobarbital metabolism is not present in all mouse-strains.

While in rats the nature of the sex difference in the rate of drug metabolism has been extensively investigated, reports on mice are scarce [6, 8]. We have investigated the effect of orchectomy, ovariectomy and pretreatment with testosterone on the rate of drug metabolism and on some components of the redox chain involved in the oxidative metabolism of drugs in mouse liver microsomes, to try to discover the factors responsible for the observed sex difference. Moreover, by comparing the effects of the treatments on the rate of drug metabolism and the components of the reaction chain, information may be obtained concerning the rate limiting factor for the oxidation of hexobarbital and other drugs.

2. Experimental

2.1. *Animals*

All mouse strains used were obtained from the T.N.O. animal breeding station in Zeist. The animals were at least ten weeks old.

2.2. *Treatments*

Orchectomy and ovariectomy and sham operations were performed under light ether anesthesia according to standard procedures, at least four weeks before the experiments.

Testosterone propionate in peanut oil (2.5 mg in 0.1 ml oil s.c./animal) was given at 14, 10, 7 and 3 days before the experiments.

2.3. *Biochemical methods*

The hydroxylation of hexobarbital and aniline and the demethylation of ethylmorphine and *N*-methylaniline were determined with the 9000 *g* liver supernatant obtained from a 33.3% homogenate in 0.1 M phosphate buffer, pH 7.4. Each incubation mixture contained 0.6 μmole NADP, 25 μmoles glucose 6-phosphate, 100 μmoles nicotinamide and 25 μmoles $MgCl_2$. For demethylation reactions 25 μmoles semicarbazide were added. Substrate concentrations were: hexobarbital 1 μmole, aniline 5 μmoles, ethylmorphine 15 μmoles and *N*-methylaniline 10 μmoles, in a total volume of 6 ml. The reaction was started by adding 1 ml of the supernatant.

The disappearance of hexobarbital was determined as described previously [9]. *p*-Aminophenol was determined according to Jaccarini et al. [10] and the formaldehyde formed in the demethylation reactions with Nash reagent as described by Cochin and Axelrod [11]. Zero order kinetics for all reactions studied was checked by determining substrate or reaction

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Table 1
The rate of hexobarbital metabolism (nmole/min) by 9000 *g* liver supernatant of male and female mice of different strains (means + S.E.M.).

Strain	CPB-SE	CPB-N	CPB-FT	CPB-V
M	14.2 ± 0.5 (6)	11.8 ± 0.8 (4)	21.6 ± 0.6 (8)	17.0 ± 0.5 (8)
F	21.0 ± 0.5 (6)	18.6 ± 0.8 (4)	23.0 ± 0.6 (8)	15.1 ± 0.5 (8)
	$P < 0.001$	$P < 0.001$	n.s.	n.s.

Table 2
The hydroxylation of hexobarbital and aniline and the demethylation of ethylmorphine and *N*-methylaniline by 9000 *g* liver supernatant of male and female CPB-SE mice (means + S.E.M.).

	Hexobarbital (nmole/min)	Aniline (nmole/min)	Ethylmorphine (nmole/min)	*N*-methylaniline (nmole/min)
M	10.1 ± 0.8 (12)	16.1 ± 0.7 (15)	173.7 ± 12.9 (12)	190.0 ± 8.6 (6)
F	19.1 ± 1.2 (12)	14.0 ± 0.6 (15)	259.3 ± 12.3 (12)	241.5 ± 10.4 (6)
	$P < 0.001$	$P < 0.05$	$P < 0.001$	$P < 0.01$

product in 1 ml of the incubate at 0, 15 and 30 min for hydroxylations and at 0, 10 and 20 min for demethylations.

Microsomal NADPH-cytochrome *c* reductase was determined as described by Williams and Kamin [12].

Microsomes for cytochrome P-450 and *b*5 determinations were prepared as described by Remmer et al. [13]. Cytochrome *b*5 was determined by addition of a few milligrams of $Na_2S_2O_4$ to the sample cuvette and cytochrome P-450 by bubbling carbon monoxide for 60 sec through the same cuvette after addition of $N_2S_2O_4$ to the reference cuvette. The microsomal suspension contained 1.5–2 mg protein/ml.

Difference spectra for hexobarbital and aniline were determined as described by Schenkman et al. [2]. The microsome suspensions contained 2.5–3 mg of protein/ml. Results are given as $A_{max-min}/\Delta A_{P\text{-}450} \times 10^{-2}$. All spectra were measured in a Unicam SP 800 B split-beam recording spectrophotometer.

Protein was determined according to Lowry et al. [14], using bovine serum albumin (BDH) as a standard.

The results were analyzed statistically by regression analysis and Student *t* test. The significance level was $P < 0.05$ two sided.

3. Results

Table 1 summarizes experiments on the metabolism of hexobarbital in the 9000 *g* liver supernatant from four mouse strains. In two strains, CPB-SE and CPB-N, female animals metabolise hexobarbital significantly faster than males, whereas in the CPB-FT and CPB-V strain no such sex difference was found. The sex difference appeared at about eight weeks after birth.

In CPB-SE mice a similar sex difference was found for the demethylation of ethylmorphine and *N*-methylaniline. However, for the hydroxylation of aniline a small opposite sex difference was found (table 2).

Castration of CPB-SE mice caused an increase in the rate of hexobarbital metabolism in male animals, which was only demonstrable from three weeks after the operation, while in females castration had no effect (table 3). Testosterone pretreatment of intact

Table 3
The influence of castration and testosterone propionate pretreatment on the rate of hexobarbital metabolism (nmole/min) by 9000 *g* liver supernatant of CPB-SE mice (means + S.E.M., n = 4).

	Males	Females
Sham	15.2 ± 2.5	26.8 ± 1.3**
Castrated	23.8 ± 0.9*	26.2 ± 1.2
Controls	11.5 ± 1.7	18.1 ± 1.3**
Testosterone	10.1 ± 1.1	11.5 ± 1.5*

* Significantly different from controls ($P < 0.05$).
** Significantly different from males ($P < 0.05$).

Table 4
The influence of castration and testosterone pretreatment on the activity of NADPH-cytochrome *c*-reductase in CPB-SE mouse liver microsomes (nmole/mg protein + S.E.M., n = 10).

	Males	Females
Sham	73.7 ± 2.8	134.6 ± 6.0**
Castrated	117.8 ± 4.9*	103.6 ± 3.9*
Control	94.0 ± 7.0	166.4 ± 11.0**
Testosterone	88.4 ± 4.0	106.2 ± 3.2*

* Significantly different from controls ($P < 0.05$).
** Significantly different from males ($P < 0.05$).

animals during two weeks caused a decrease in the rate of hexobarbital metabolism in females; in males no significant effect was found (table 3).

The activity of NADPH-cytochrome *c* reductase was greater in liver microsomes of female CPB-SE mice. Testosterone pretreatment caused a significant decrease in the activity of this enzyme in female, but not in male animals (table 4). Castration diminished this sex difference but did not completely abolish it (table 4). Moreover, in females a decrease in the enzyme activity was found after castration. Therefore no parallellism exists between the activity of this enzyme and the rate of hexobarbital metabolism.

The effects of castration and pretreatment with testosterone propionate on the amount of cytochrome P-450 and *b*5 and the magnitude of the difference spectra caused by addition of hexobarbital and aniline in CPB-SE mice are summarized in table 5. The amounts of cytochrome P-450 in liver microsomes of male and female CPB-SE mice differ significantly in these experiments. Orchectomy equalizes the amounts of cytochrome P-450, although the pretreated group does not differ significantly from the controls. Ovariectomy had no influence. Testosterone pretreatment decreases the cytochrome P-450 levels both in male and in female animals.

No sex difference was observed in the amount of microsomal cytochrome *b*5. Castration as well as testosterone pretreatment had no effect. The magnitude of the aniline difference spectrum did not differ in males and females; both castration and testosterone administration were without effect. The magnitude of the difference spectrum for hexobarbital was smaller in males, however, orchectomy did not produce an increase. Pretreatment with testosterone decreased the difference spectrum in female animals.

4. Discussion

The present investigations show that in some mouse strains hexobarbital is metabolised faster by female than by male animals. The sex difference appears when the animals are about eight weeks old. In other strains this sex difference was not demonstrated. This phenomenon was further investigated in the CPB-SE strain. It was found that a similar sex difference exists for the demethylation of ethylmorphine and *N*-methylaniline. The first substance, like hexobarbital, is a type I substrate [8] but the latter, like aniline, is a type II substrate in rabbits [15], which we could confirm for mice. Therefore no relation exists between the type of the substrate and the presence or absence of the sex difference mentioned in its rate of metabolism.

In order to investigate which hormone may be responsible for this phenomenon, male and female animals were castrated. This caused an increase in the rate of hexobarbital metabolism in males, which developed during four weeks after the operation. In female animals no effect of castration was observed. This finding suggested a role of testosterone. It was found indeed that administration of the propionate of this hormone during two weeks caused a decrease

Table 5

The influence of castration and testosterone pretreatment on the amounts of cytochrome P-450 and *b*5, the magnitude of the hexobarbital and aniline-induced difference spectra and the protein content of liver microsomes of CPB-SE mice (means ±S.E.M.).

	Cyt. P-450 $A_{450-500}$/mg prot	Cyt. *b*5 $A_{425-450}$/mg prot	Diff. spectrum hexob./P-450	Diff. spectrum aniline/P-450	mg protein/g liver
M sham	123.0 ± 6.0 (12)	76.4 ± 5.7 (12)	12.3 ± 1.4 (8)	30.6 ± 2.4 (8)	16.6 ± 0.8 (12)
M castr.	138.9 ± 7.1 (12)	93.4 ± 7.3 (12)	12.5 ± 1.6 (8)	31.2 ± 2.6 (8)	15.5 ± 0.5 (12)
F sham	148.0 ± 7.5** (12)	90.4 ± 4.2 (12)	24.4 ± 1.6** (8)	28.1 ± 1.5 (8)	15.4 ± 0.7 (12)
F castr.	148.0 ± 5.7 (12)	92.5 ± 8.1 (12)	21.7 ± 2.7 (8)	32.1 ± 3.1 (8)	15.3 ± 0.4 (12)
M control	118.0 ± 3.6 (8)	57.9 ± 4.1 (8)	6.6 ± 0.9 (8)	28.5 ± 1.3 (8)	17.2 ± 0.5 (8)
M testost.	107.3 ± 3.0* (8)	54.8 ± 3.1 (8)	9.1 ± 0.8 (8)	28.4 ± 1.5 (8)	18.7 ± 0.8 (8)
F control	131.9 ± 3.6** (8)	62.2 ± 3.0 (8)	21.9 ± 0.9** (8)	25.9 ± 0.7 (8)	16.8 ± 1.0 (8)
F testost.	105.0 ± 2.2* (8)	58.1 ± 2.1 (8)	9.3 ± 0.7* (8)	29.9 ± 1.6 (8)	18.5 ± 0.4 (8)

* Significantly different from controls ($P < 0.05$).
** Significantly different from males ($P < 0.05$).

of the rate of hexobarbital metabolism in female animals, but was without effect in males. Apparently the time interval of three days between the last testosterone injection and the experiments was sufficient for the testosterone to be eliminated from the body. Otherwise a competitive inhibition of the hexobarbital metabolism was to be expected [16].

The mechanism of action of testosterone was further investigated by determining the effect of castration and testosterone administration on some components of the redox chain involved in microsomal drug metabolism and comparing these with the effects on the rate of hexobarbital metabolism in male and female CPB-SE mice. It is likely that, if some factor changes parallel with the total enzyme activity, it is rate limiting for the hydroxylation of hexobarbital.

NADPH-cytochrome *c* reductase activity was higher in liver microsomes of females. Testosterone pretreatment reduced this activity only in females. However, ovariectomy decreased the enzyme activity but not the rate of hexobarbital hydroxylation. Therefore the observed sex difference in the activity of this enzyme does not explain the sex difference in the rate of hexobarbital metabolism. The amount of cytochrome P-450 was found to be higher in liver microsomes of female animals than in males. This difference was abolished by castration. Testosterone administration lowered the P-450 levels, both in males and females, contrary to its effects on the rate of hexobarbital metabolism. Moreover Davies et al. [8] reported an opposite sex difference of the cytochrome P-450 levels in liver microsomes of a mouse strain in which female animals metabolise ethylmorphine faster than males, like our CPB-SE strain. Therefore it is unlikely that the total amount of cytochrome P-450 in liver microsomes determines the rate of hexobarbital metabolism. Nevertheless a rate limiting role of one form of cytochrome P-450 instead of the total content cannot be excluded.

The type I difference spectrum of hexobarbital is stronger in female mice, even if calculated per unit of cytochrome P-450. However, orchectomy does not increase its magnitude, although testosterone pretreatment decreases it in female animals. Therefore the sex difference in the rate of hexobarbital metabolism cannot be explained by the observed sex difference in the hexobarbital difference spectrum. The same applies for the amount of microsomal cytochrome *b*5. The amount of this cytochrome did not differ in males and females, whereas castration and testosterone pretreatment had no influence on this parameter.

The conclusion from these experiments is that none of the observed sex differences in the components of the microsomal drug metabolising enzyme system can be responsible for the sex difference in the rate of drug metabolism in this mouse strain.

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