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Unresponsiveness of female rat hepatic monooxygenases to physiological levels of testosterone

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Biotransformation of a wide variety of xenobiotics and endogenous compounds is accomplished by a class of microsomal hemoproteins known as the cytochrome P-450s [1, 2]. A copious body of compelling evidence is now available demonstrating the existence of sex differences in the hepatic metabolism of drugs in rats [1, 3, 4]. In general, males exhibit higher rates of metabolic conversion of various substrates including hexobarbital, aminopyrine and ethylmorphine than do females [1, 3, 4]. This sex difference in metabolism has been attributed to the stimulatory effects of androgen, since orchidectomy increases the plasma half-life of these compounds with a concomitant decrease in the activities of hepatic monooxygenases, whereas testosterone treatment results in a reversal of this castration effect [1, 3, 4]. In a similar vein, past reports have shown that androgen exposure in the neonatal period is also important, imprinting a basic male pattern of metabolism and an ability to respond to androgens later in life [4, 5]. In contrast, neonatal or adult ovariectomy has little effect on the hepatic monooxygenases, indicating that estrogen is not necessary for the imprinting or maintenance of the female type of drug metabolism [3–5].

Unfortunately, most studies of androgenic regulation of drug metabolism have used suprapharmacological doses of androgens and, thus, any sex-related differences in the responsiveness of hepatic monooxygenases to physiological levels of these hormones are unknown. In the present study we have examined the effects of a physiological dose of testosterone on the hepatic monooxygenase system of gonadectomized male and female rats.

Materials and methods

Animals. Three-month-old male and female Sprague–Dawley rats [Crl:CD(SD)BR, Charles River Breeding Laboratories, Inc., Wilmington, MA] were maintained under standardized conditions of light (7:00 a.m.–7:00 p.m.) and temperature (20–23°) on a diet of laboratory chow and water. Animals were housed 2–3 to a cage on hardwood bedding chips.

Treatments. Male rats were castrated through scrotal incisions and female rats were ovariectomized by a dorsal paralumbar approach [6]. All control animals were sham operated. Two weeks after surgery, half of the gonadectomized animals were injected subcutaneously with testosterone propionate (TP, 0.20 mg/kg daily), while the remaining castrates and all of the sham-operated rats received an equivalent amount of the corn oil vehicle for the 45-day treatment period. Rats were rapidly killed by decapitation between 9:00 and 10:00 a.m. on the day following the last injection.

Livers were perfused with ice-cold 0.9% saline and then removed, trimmed, weighed, minced into small pieces, and homogenized in 2 vol. of cold 0.25 M sucrose containing 50 mM Tris–HCl (pH 7.4). The homogenate was treated as described previously [7].

Hexobarbital hydroxylase activity was determined by our modification [6] of the radioenzyme procedure of Kupfer and Rosenfeld [8]. The assay measures the rate of microsomal conversion of radioactive hexobarbital, 5-[2-^{14}C]cyclohexenyl-3,5-dimethylbarbituric acid (14.4 mCi/mmol; NEN Research Products, Boston, MA), to 3-hydroxyhexobarbital. Linear kinetic data about the enzyme were obtained using ten different hexobarbital concentrations (0.035 to 0.4 mM). Apparent Michaelis constants (K_m) and maximal velocities (V_{max}) were obtained from linear regression models of the data using the method of Hofstee [9]. The correlation coefficients for all Hofstee plots were positive, exceeded 0.95, and were statistically significant ($P < 0.05$).

Total cytochrome P-450 was quantified by measuring the carbon monoxide difference spectra after reduction with dithionite [10]. Cytochrome b_5 and total microsomal heme were measured according to the procedures of Omura and Sato [10] and Falk [11] respectively. Microsomal protein was determined by the method of Lowry *et al.* [12] with bovine serum albumin as the standard.

Quantitation of serum testosterone levels was carried out by our previously reported method [13] using a specific antiserum obtained from Radioassay Systems Laboratories, Inc. (Carson City, CA).

Experimental groups were compared by analysis of variance and Student's *t*-test.

Results and discussion

The prolonged period of orchidectomy produced expected [1, 3, 14] but profound decreases in ventral prostate and seminal vesicle weights and concentrations of serum testosterone (Table 1), as well as declines to female-like levels of hexobarbital hydroxylase, cytochromes P-450 and b_5, and heme (Table 2). Daily administration of 200 μg/kg of testosterone increased the serum concentration of the hormone in the orchidectomized rats to intact values. Concomitantly, the restoration of physiological levels of serum testosterone increased the weights of the ventral prostate and seminal vesicles and the concentrations of hepatic hexobarbital hydroxylase, cytochromes P-450 and b_5, and heme to the pre-castration levels. As expected [1, 3, 15], gonadectomy in the female rats produced no alterations in the activities of the hepatic monooxygenases and the concentrations of hepatic heme (Table 2). In contrast to the male, daily treatment with 200 μg/kg of testosterone had no effect on the levels of hepatic hexobarbital hydroxylase, cytochromes P-450 and b_5, and heme, in spite of the fact that the serum concentration of testosterone was increased to male-like values (Table 1).

Our data concerning sex differences in cytochrome P-450 and hexobarbital hydroxylase are in agreement with previously reported results [1, 3]. It is noteworthy, however, that the hepatic monooxygenase system in the gonadectomized female rats was unresponsive to the action

Table 1. Body and organ weights and serum testosterone levels of intact, castrated and testosterone propionate (TP)-treated male and female rats

	Body weight (g)	Ventral prostate (mg/100 g body weight)	Seminal vesicles (mg/100 g body weight)	Testosterone (ng/ml serum)
Males				
Intact	557 ± 22	126.9 ± 7.5	106.8 ± 5.4	2.11 ± 0.21
Castrated	523 ± 15	9.6 ± 1.2*	16.3 ± 1.9*	0.31 ± 0.04*
Castrated + TP	594 ± 14†	110.9 ± 15.0‡	92.6 ± 8.9‡	1.95 ± 0.28‡
Females				
Intact	310 ± 5			0.15 ± 0.02
Castrated	392 ± 9*			0.06 ± 0.02§
Castrated + TP	396 ± 11*			1.83 ± 0.20*‡

All values are expressed as mean ± SEM for eight rats/groups.
*§ Significantly different when compared to intacts of the same sex: *$P < 0.001$ and §$P < 0.01$.
†‡ Significantly different when compared to castrates of the same sex: †$P < 0.01$, and ‡$P < 0.001$.

Table 2. Hepatic heme and monooxygenase enzymes of intact, castrated and testosterone propionate (TP)-treated male and female rats

	Hexobarbital hydroxylase		Cytochrome		
	V_{max} (nmol/min/mg protein)	K_m (mM)	P-450	b_5 (nmol/mg protein)	Heme
Males					
Intact	11.9 ± 0.8	0.22 ± 0.01	1.03 ± 0.06	0.27 ± 0.03	1.72 ± 0.12
Castrated	3.3 ± 0.3*	0.20 ± 0.02	0.67 ± 0.02*	0.20 ± 0.01†	1.22 ± 0.05*
Castrated + TP	9.7 ± 1.0‡	0.21 ± 0.02	0.93 ± 0.04‡	0.25 ± 0.02§	1.58 ± 0.03‡
Females					
Intact	2.4 ± 0.2	0.20 ± 0.02	0.74 ± 0.02	0.21 ± 0.02	1.35 ± 0.05
Castrated	2.2 ± 0.2	0.19 ± 0.02	0.67 ± 0.03	0.18 ± 0.02	1.27 ± 0.06
Castrated + TP	2.8 ± 0.3	0.20 ± 0.02	0.71 ± 0.03	0.21 ± 0.01	1.36 ± 0.06

All values are expressed as mean ± SEM for eight rats/group.
*† Significantly different when compared to intacts of the same sex: *$P < 0.001$, and †$P < 0.04$.
‡§ Significantly different when compared to castrates of the same sex: ‡$P < 0.001$, and §$P < 0.04$.

of exogenously administered testosterone. To our knowledge, this phenomenon has not been reported previously. In fact, a number of investigators have shown that a male-type of hepatic metabolism can be established in female rats upon the administration of exogenous testosterone [1, 3, 15]. The reason for the seemingly discrepant results is more likely to be due to the dose of testosterone used—suprapharmacological doses in past reports, in contrast to a physiological dose used in our study. In fact, we have reported recently [16, 17], in agreement with our present findings, a similar sexually dimorphic response to phenobarbital induction. In contrast to male rats, daily treatment of female rats with either 1 or 3 mg/kg of phenobarbital produces no stimulatory effects on the activities of the hepatic monooxygenases. When the barbiturate is administered at a considerably higher level, both sexes exhibit similar magnitudes of induction.

The reason for the insensitivity of hepatic monooxygenases in female rats to physiological levels of androgen is unknown. However, this sexually dimorphic response to testosterone is probably predetermined or "imprinted" during a critical developmental period, since perinatal exposure to androgens [4, 5] can alter permanently the sexual nature of the inductive response. Previous studies have reported, however, that androgens do not act directly on the liver, but rather affect the pulsatile secretion of pituitary growth hormone which, in turn, regulates the sex differences in hepatic monooxygenases [18, 19]. In this regard, sexual dimorphisms have been observed in the secretory patterns of hypothalamic growth hormone releasing factor and the inhibitory factor, somatostatin, which together regulate the pulse rate of circulating growth hormone [19]. Therefore, it is not unreasonable to suggest that the hepatic monooxygenases of female rats are unresponsive to physiological doses of testosterone because their hypothalamic centers regulating growth hormone secretion are insensitive to "normal" serum levels of the androgen.

In summary, we report that the sensitivity of the hepatic monooxygenase system to androgen induction was sex dependent in rats. In contrast to males, the hepatic enzymes in females were unresponsive to physiological levels of testosterone.

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