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Sex-related difference in oxidative metabolism of testosterone and erythromycin by hamster liver microsomes

Toshiaki Miura, Masayuki Komori, Miyuki Iwasaki, Kimikazu Kurozumi, Kazuhide Ohta, Shigeru Ohmori[+], Mitsukazu Kitada[+] and Tetsuya Kamataki

Division of Analytical Biochemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060 and *[+]Faculty of Pharmaceutical Sciences, Chiba University, Chiba 260, Japan*

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The activities of testosterone hydroxylases and erythromycin *N*-demethylase were significantly higher in liver microsomes from female hamsters than in the male counterparts. SDS-polyacrylamide gel electrophoresis revealed a difference in protein composition between male and female liver microsomes in the molecular mass region comprising cytochrome P-450. Western blot analysis showed further that antibodies to rat male-specific cytochrome P-450 crossreacted with at least two proteins in both male and female hamster microsomes, but one of the female proteins had a different molecular mass from that of the male proteins. It is concluded that sex difference in liver microsomal cytochrome P-450 is not restricted to rats and mice, as has previously been believed.

Sex difference; Cytochrome P-450; Testosterone hydroxylase; Erythromycin *N*-demethylase; (Hamster, Liver microsome)

1. INTRODUCTION

Multiple forms of cytochrome P-450 occur in liver microsomes and display distinct but broad and overlapping substrate specificities [1]. They play a crucial role in oxidative metabolism of various drugs and endogenous substrates such as steroids. Recent studies have established the existence of sex-related difference in the oxidative metabolism by liver microsomes of rats and limited strains of mice [2–4]. This difference has been accounted for in part by the presence of sex-specific forms of cytochrome P-450, the expression of which is modulated by sex hormones via growth hormone secretion [5–11]. However, no such sex difference has been reported in animals other than the aforementioned species and it is generally held that the sex difference is restricted to rats and mice [2]. We report here that sex difference does exist in cytochrome P-450-dependent drug and steroid oxidations by hamster liver microsomes.

Correspondence address: T. Miura, Faculty of Pharmaceutical Sciences, Hokkaido University, Nishi-6-chome, Kita-12-jo, Kita-ku, Sapporo 060, Japan

2. MATERIALS AND METHODS

Male and female Syrian golden hamsters (8 weeks of age) were obtained from Sankyo (Tokyo) and killed by decapitation. Liver microsomes were prepared as described in [12]. Protein was determined by the method of Lowry et al. [13]. The contents of cytochromes P-450 and b_5 were estimated as described by Omura and Sato [14,15]. Steroid and drug oxidizing activities were assayed using an incubation mixture, which contained (in a total volume of 1.0 ml) liver microsomes (about 0.5 mg protein), 0.05 mM EDTA, 100 mM phosphate buffer (pH 7.4), a substrate (0.6 mM testosterone, 0.6 mM progesterone, 5 mM aminopyrine, 5 mM ethylmorphine, 5 mM benzphetamine or 0.6 mM erythromycin) and an NADPH-generating system (5 mM $MgCl_2$, 0.5 mM $NADP^+$, 5 mM glucose 6-phosphate and 1 unit of glucose 6-phosphate dehydrogenase). After 5 min preincubation at 37°C, the reaction was started by adding the NADPH-generating system. The reaction was run for 15 min at 37°C and terminated by adding 6 ml of benzene (for steroid oxidations) or 100 μl of 10% trichloroacetic acid (for drug oxidations). Drug *N*-demethylase activities were assayed by determining formaldehyde formed [16]. For determination of steroid hydroxylase activities, the benzene extract was evaporated to dryness under reduced pressure. The residue was dissolved in 200 μl of methanol containing an internal standard (cortisol acetate for testosterone hydroxylases and prednisone for progesterone hydroxylases). An aliquot of the methanol solution was applied to a high-performance liquid chromatograph (Toyo Soda model HLC-803D) equipped with a UV absorbance detector (Shimadzu Model SPD-2A) and a

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gradient unit (Toyo Soda model GE-4). The chromatographic conditions described by Hayashi et al. [17] were used for detection of testosterone metabolites. For detection of progesterone metabolites, reverse-phase chromatography was performed on an Inertsil ODS column (∅ = 5 μm, 4.6 × 250 mm, Gasukuro Kogyo Inc.) preceded by a guard column (4.6 × 50 mm). The metabolites were separated at a flow rate of 1.0 ml/min and by isocratic and gradient elution as follows; from 0 to 22 min with A followed by a linear gradient to B at 35 min (A, acetonitrile/water, 4:6, by vol.; B, acetonitrile/water, 7:3, by vol.). The effluent was monitored at 240 nm. SDS-polyacrylamide gel electrophoresis was conducted by the method of Laemmli [18] using 7.5% cross-linked gel (0.75 mm thick and 10 cm long). The gel was silver stained. Western blot analysis was performed by the method of Towbin et al. [19] as modified by Guengerich et al. [20] using antibodies to P-450-male, a male-specific form of rat liver microsomal cytochrome P-450 [6].

3. RESULTS AND DISCUSSION

Fig.1 shows high-performance liquid chromatograms of metabolites produced from testosterone and progesterone by male and female hamster liver microsomes. In confirmation of earlier observations [21], male microsomes formed 2β-, 6β- and 7α-hydroxytestosterones and an unidentified product from testosterone as major metabolites. Female microsomes also yielded the same products from testosterone. However, the formation of the 2β- and 6β-hydroxytestosterone and the unidentified product was significantly higher in female microsomes, whereas that of 7α-hydroxytestosterone was higher in male microsomes (table 1). On the other hand, no apparent sex difference was observed in the metabolism of progesterone. Both male and female microsomes produced 6β-, 15β- and 16α-hydroxyprogesterones to approximately the same extents (table 1). We next examined drug oxidizing activities of and specific contents of cytochromes P-450 and b_5 in the two types of liver microsomes. As shown in table 1, no sex difference was detected in aminopyrine, ethylmorphine and benzphetamine *N*-demethylase activities as well as the cytochrome contents. However, erythromycin

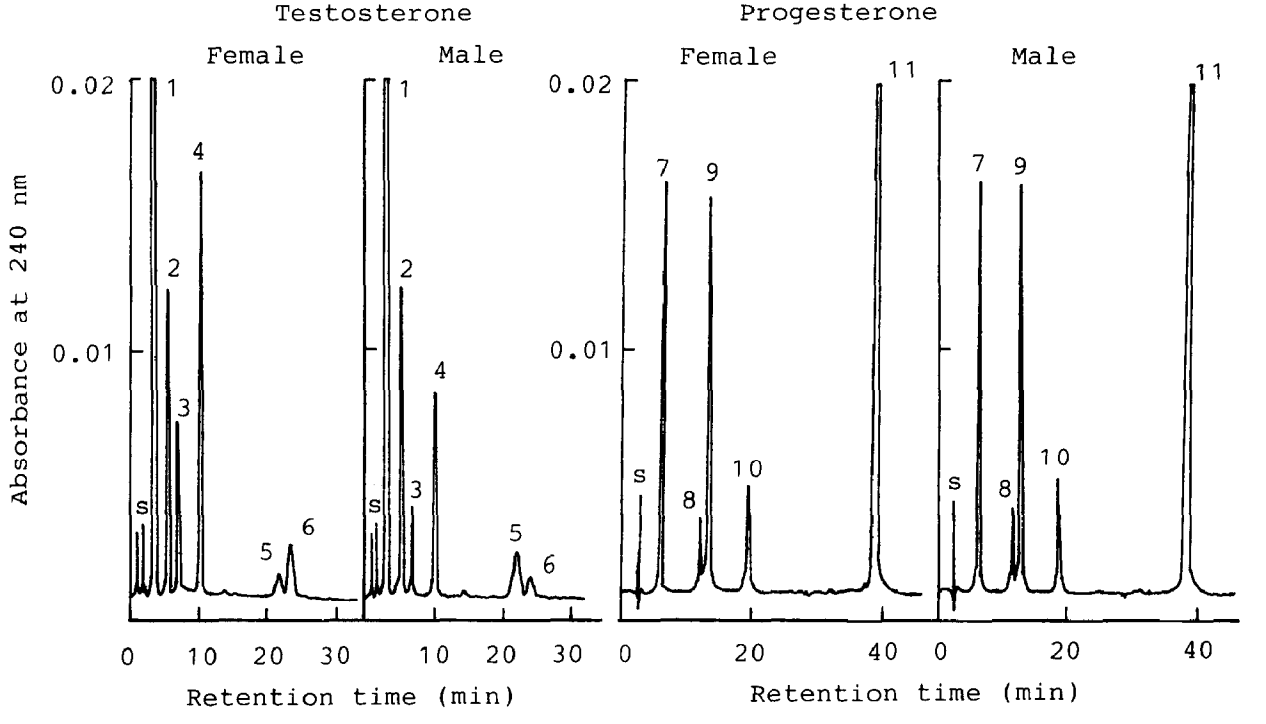

Fig.1. High-performance liquid chromatograms of testosterone and progesterone metabolites formed by liver microsomes from female and male hamsters. Concentrations of microsomal protein were 0.319 and 0.318 mg per ml of incubation mixture for male and female, respectively. Incubations were carried out as described in section 2. s, solvent; 1, testosterone; 2, cortisol acetate (internal standard); 3, 2β-hydroxytestosterone; 4, 6β-hydroxytestosterone; 5, 7α-hydroxytestosterone, 6, unidentified product; 7, prednisone (internal standard); 8, 16α-hydroxyprogesterone; 9, 15β-hydroxyprogesterone; 10, 6β-hydroxyprogesterone; 11, progesterone.

N-demethylase activity of female microsomes was significantly higher than that of male microsomes.

Since sex difference was detected in the activities of testosterone hydroxylases and erythromycin *N*-demethylase but not in the cytochrome P-450 content, it was likely that a difference exists in the relative proportion of forms of cytochrome P-450 between male and female hamster liver microsomes. To test this possibility, we examined the protein composition of both types of microsomes by SDS-polyacrylamide gel electrophoresis. As shown in fig.2, sex difference was clearly observed in protein bands in the molecular mass region comprising different forms of cytochrome P-450 (50–60 kDa). Fig.2 also shows the results of Western blot analysis of microsomal proteins using antibodies to P-450-male, a rat sex-specific form of cytochrome P-450 [6]. Both male and female hamster liver microsomes contained at least two forms of cytochrome P-450 that cross-reacted with the antibodies. One of them possessed the same apparent molecular mass (52 kDa) as P-450-male and was common to male and female. The other form in male or female microsomes had a lower or higher molecular mass than P-450-male, respectively. These observations provide a support to the view that sex-specific forms of cytochrome P-450 do exist in hamster liver microsomes.

The expression of male- and female-specific forms of cytochrome P-450 in rats has been reported to be regulated by sex hormones through affecting the secretion pattern of growth hormones [4,8,10,11]. Colby et al. [22] have shown that corticosterone metabolism in male hamsters was affected by gonadectomy and by testosterone treatment. In male hamsters it has also been reported that aryl hydrocarbon hydroxylase activity of kidney microsomes was responsive to both estrogens and androgenic hormones [23]. It is, therefore, reasonable to assume that the expression

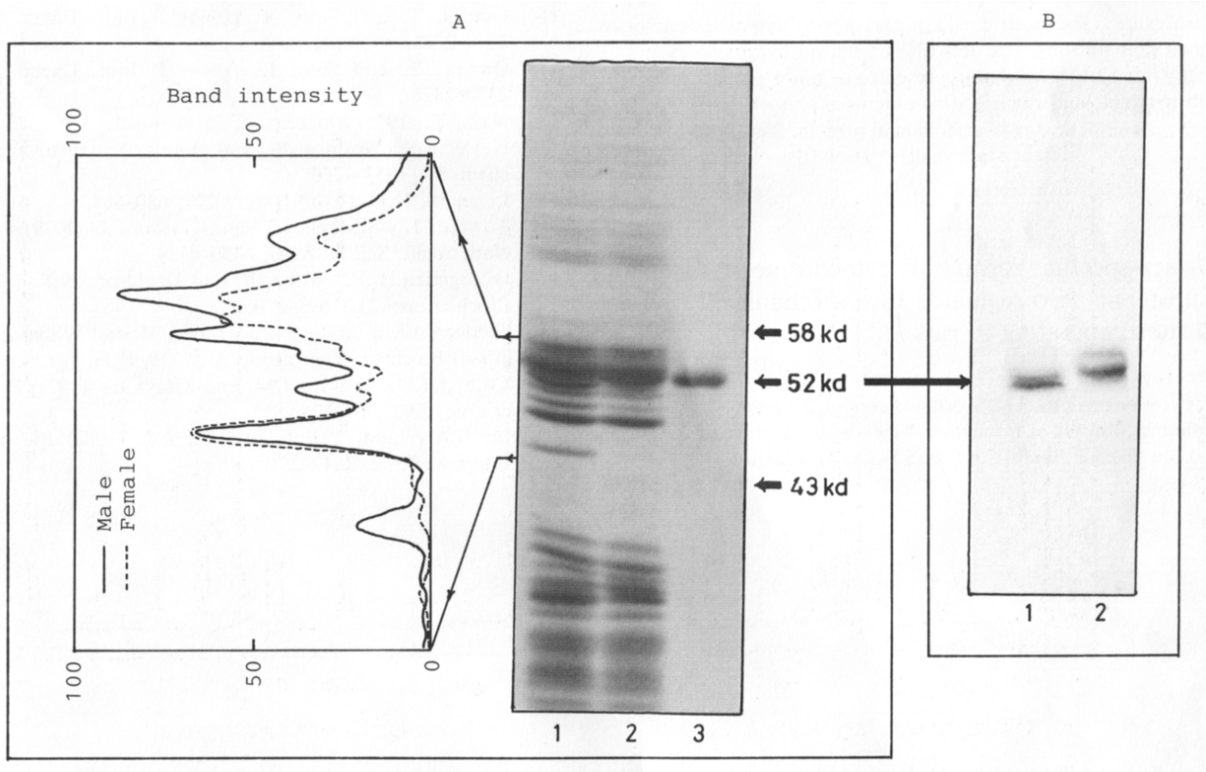

Fig.2. SDS-polyacrylamide gel electrophoresis profiles (A) and Western blot analysis using anti-P-450-male antibodies (B) of proteins in liver microsomes from male and female hamsters. The intensities of protein bands in the regions of molecular mass of cytochrome P-450 were measured by using a densitometer (Quick Scan R & D, Helena, Beaumont, USA). Lanes: 1, liver microsomes from male hamsters; 2, liver microsomes from female hamsters; 3, purified cytochrome P-450-male.

Table 1

Steroid hydroxylase and drug *N*-demethylase activities, and specific contents of cytochromes P-450 and b_5 in liver microsomes from male and female hamsters

Measurement	Male	Female
Testosterone hydroxylase ($n = 5$)		
2β-OH	1.47 ± 0.22	2.31 ± 0.12*
6β-OH	5.92 ± 1.10	7.80 ± 0.55*
7α-OH	1.29 ± 0.29	0.55 ± 0.12*
Unknown	1.00 ± 0.13	1.56 ± 0.07*
Progesterone hydroxylase ($n = 5$)		
6β-OH	1.36 ± 0.29	1.70 ± 0.42
15β-OH	3.33 ± 0.52	3.89 ± 1.23
16α-OH	0.41 ± 0.07	0.44 ± 0.07
Drug *N*-demethylase ($n = 6$)		
Ethylmorphine	26.5 ± 4.2	25.4 ± 1.7
Aminopyrine	28.0 ± 3.0	27.1 ± 2.2
Benzphetamine	10.9 ± 0.9	10:6 ± 0.7
Erythromycin	4.43 ± 0.55	5.94 ± 0.52*
Specific content ($n = 6$)		
Cytochrome P-450	1.10 ± 0.07	1.06 ± 0.14
Cytochrome b_5	0.48 ± 0.03	0.47 ± 0.04

Each value is the mean ± SD of assays with five or six microsomal preparations. The activities of steroid hydroxylases and drug *N*-demethylases were represented as nmol per mg of protein per min, and the specific contents of cytochromes P-450 and b_5 as nmol per mg of microsomal protein. Significantly different from male (* $P<0.01$)

of sex-specific forms of cytochrome P-450 in hamsters is also regulated by a mechanism similar to those operating in rats.

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