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# Aromatization of 7α-Methyl-19-nortestosterone by Human Placental Microsomes *In Vitro*

**Annette LaMorte,* Narender Kumar, C. Wayne Bardin and Kalyan Sundaram†**

*Center for Biomedical Research, The Population Council, 1230 York Avenue, New York, NY 10021, U.S.A.*

**Part of the biological effects of testosterone (T) are mediated by its enzymatic reduction to 5α-dihydrotestosterone (DHT) or aromatization to estradiol ($E_2$). 7α-Methyl-19-nortestosterone (MENT) is a synthetic androgen that is considerably more potent than T. Previous studies have shown that MENT is not 5α-reduced. The studies reported here were undertaken to determine whether MENT undergoes enzymatic aromatization *in vitro*. Human placental microsomes were used as the source of the aromatase. Radioactive or nonradioactive T or MENT was incubated with the microsomes in the presence of NADPH and the metabolites extracted out with ethyl ether. Following evaporation of ether, the residue was dissolved in benzene–petroleum ether and extracted with 0.4 N NaOH which selectively removes phenolic metabolites of the androgens. When either radioactive T or MENT was incubated with the aromatase in the presence of NADPH, there was a 20-fold increase in the amount of radioactivity extracted with NaOH. In contrast, if the incubation was carried out in the absence of NADPH or in the presence of R76713, an aromatase inhibitor, most of the radioactivity remained in the benzene–petroleum ether phase. To further identify the enzymatic reaction products, thin layer chromatography (TLC) was performed. The $R_f$ value for MENT was 0.22 while that of the major reaction product was 0.34, which corresponded with the RF value of the estrogen, 7α-methyl-estradiol ($MeE_2$). This was further verified by using a second solvent system for the chromatographic separation. In an effort to ascertain whether the metabolites bind to estrogen receptors (ER), rat uterine cytosol was used. NaOH extracts of medium following incubation of nonradioactive MENT with microsomes showed competitive inhibition of [$^3$H]$E_2$ binding to rat uterine ER. Furthermore, after [$^3$H]MENT was incubated with microsomes, the radioactive metabolite extracted in NaOH showed specific binding to the ER which could readily be displaced with $E_2$ or $MeE_2$. These results indicate that like T, MENT undergoes enzymatic aromatization.**

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## INTRODUCTION

Testosterone (T), the principal androgen secreted by the Leydig cells acts on most body tissues by interacting with specific androgen receptors. In some tissues, part of the important biological actions of T depend upon its enzymatic conversion to active metabolites [1]. In the male accessory organs and skin, T undergoes 5α-reduction to 5α-dihydrotestosterone (DHT) which leads to an amplification of its androgenic potency [2, 3]. In some parts of the brain and in adipose tissue T is converted to estradiol ($E_2$) via the enzyme aromatase [4–6]. Hence, the biological actions of T are complex and the tissue response depends upon the enzymatic activity of the target tissues and the active intracellular hormone. 7α-Methyl-19-nortestosterone (MENT) is a synthetic androgen whose biological potency is several-fold higher than that of T [7]. Compared to T, the relative potency of MENT on the prostate and seminal vesicles is 4–5 times higher, while its potency on the muscle and pituitary is 10–12 times higher [8]. The differential potency of MENT on the sex accessory organs and muscle can be attributed to the finding that MENT cannot be 5α-reduced [9]. As a consequence, administration of a 5α-reductase inhibitor decreased

*Present address: Department of Obstetrics and Gynecology, Yale University Medical School, New Haven, CT 06510, U.S.A.
†Correspondence to K. Sundaram.
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the response of the prostate to T but not to MENT or DHT [8]. The resistance of MENT to 5α-reduction confers a special advantage on it for use as a replacement androgen in that a dose of MENT sufficient to maintain normal action on muscle and other organs will underreplace the prostate. This could possibly lead to a reduced incidence of prostatic hypertrophy in individuals who receive MENT.

Since T undergoes enzymatic aromatization to $E_2$, a number of reports have attributed a variety of roles for $E_2$ in the male, including the regulation of gonadrotropin secretion and sexual behavior [10–12]. Therefore, the present studies were undertaken to determine whether MENT could be aromatized. Human placental microsomes were used as the source of aromatase.

## EXPERIMENTAL

### *Chemicals and isotopes*

All solvents were analytical reagent grade. Biochemicals were obtained from Sigma Chemical Company (St Louis, MO). [1β-$^3$H]Androstenedione (27 Ci/mmol), [1,2,6,7-$^3$H]T (81 Ci/mmol) and [2,4,6,7-$^3$H]$E_2$ (110 Ci/mmol) were purchased from New England Nuclear Labs (Boston, MA). [6,7-$^3$H]MENT (20 Ci/mmol) was custom synthesized by New England Nuclear. The aromatase inhibitor, R76713 (6-[(4-chlorophenyl)(1H - 1,2,4 - triazol - 1 - yl)methyl] - 1-methyl-1H-benzotriazole), was a gift from Dr C. R. Bowden (Janssen Research Foundation, Spring House, PA).

### *Preparation of human placental microsomes*

Washed placental microsomes were prepared according to the method of Taniguchi *et al.* [13]. Human term placentas were obtained within 1 h of delivery and were kept frozen at −70°C. Placenta was thawed to 4°C, cleared of extraneous tissue and large blood vessels, washed with 1.15% KCl, and minced. The minced tissue was homogenized with 2 vol of 10 mM potassium phosphate buffer (pH 7.4) containing 0.14 M KCl and 10 mM EDTA, and centrifuged at 1000 ***g*** for 10 min. The supernatant was centrifuged at 10,000 ***g*** for 30 min and the pellet discarded. The supernatant was recentrifuged at 105,000 ***g*** in a Beckman ultracentrifuge for 90 min. Pellet containing the microsomes was resuspended in 100 mM potassium phosphate buffer (pH 7.4) containing 1 mM EDTA and 2 μM androstenedione using a glass homogenizer and was recentrifuged at 105,000 ***g*** for 90 min. The pellet was reconstituted in a small volume of 50 mM phosphate buffer (pH 7.4) containing 100 mM KCl, 20% glycerol, 2 μM androstenedione and 1 mM dithiothreitol (DTT), and small aliquots were stored at −70°C. Protein concentration was determined by using the Pierce BCA Protein Assay Reagent® and bovine serum albumin served as the standard.

The presence of aromatase activity in the microsome preparation was validated by the procedure described by Thompson and Siiteri [14] and depends on the production of $^3H_2O$ from added [1β-$^3$H]androstenedione. Aromatization of [1β-$^3$H]androstenedione leads to the production of estrone and $^3H_2O$. The incubation conditions were as follows:

Final incubation volume was 1 ml. To 50 mM potassium phosphate buffer (pH 7.4), [1β-$^3$H]androstenedione (0.5 μCi, 18 pmol), 12 μmol of glucose-6-phosphate, 2 μmol of $NADP^+$ and 2 U of glucose-6-phosphate dehydrogenase were added. The mixture was incubated for 10 min in a shaking water bath at 37°C to generate NADPH. Microsomal protein was then added to start the enzyme reaction and the incubation continued for 60 min. The reaction was stopped by the addition of 4 ml ethyl ether to each tube and vigorous mixing for 60 s. The aqueous phase was frozen in an acetone-dry ice bath and the ether phase was decanted and discarded. The ether extraction was repeated. An aliquot of the aqueous phase was placed in a scintillation vial and 5 ml of Ready Safe™ (Beckman) scintillation fluid was added and the radioactivity counted in a liquid scintillation counter (Packard).

### *Incubation of [$^3$H]T and [$^3$H]MENT with placental microsomes*

*Thin layer chromatography (TLC) of reaction products.* Tritiated androgens (0.5 μCi of [$^3$H]T or [$^3$H]MENT) were incubated with 80–160 μg of placental microsomes for 60 min and the products were extracted twice with 4 ml ethyl ether as described above. The extracts were evaporated and the residues dissolved in 100 μl acetone. Aliquots were applied to silica gel coated TLC plates. Radioactive T, $E_2$ and MENT were used as standards. In addition, nonlabeled 7α-methyl-estradiol ($MeE_2$) was used as standard because of nonavailability of radiolabeled $MeE_2$. The TLC plates were developed using methylene chloride–ethyl acetate (8:2, v/v) as mobile phase, and the radioactivity on the plate was located using a system 200 BIOSCAN imaging scanner (BIOSCAN, Washington, DC). Nonlabeled $MeE_2$ was located by using ultraviolet absorption. Experiments were done with and without R76713 during incubation.

*Separation of products by solvent partitioning.* Incubations and extractions were carried out as described above and the neutral and phenolic fractions were separated by partitioning between organic and aqueous (NaOH) phases [15]. Briefly, the residue from the ether extract following incubation of the androgens with microsomes was dissolved in 5 ml of benzene–petroleum ether (BP) (1:1, v/v) and extracted with 1 ml of 0.4 N NaOH three times and the extracts pooled. The radioactivity in aliquots of the BP and NaOH was measured. The base extract was neutralized with an equal volume of 0.4 N HCl to reduce quenching during measurement of radioactivity. In addition, the metabolites in the NaOH were extracted with ethyl ether, concentrated and fractionated on TLC using chloroform–toluene–methanol (14:7:2, by vol) as the mobile phase and scanned for radioactivity.

In another experiment, the residue from the ether extract following incubation of the androgens with microsomes was treated with sodium borohydride as described by Sisenwine *et al.* [16], and Barbieri *et al.* [17]. Briefly, the residue was reacted with $NaBH_4$ in aqueous ethanol followed by the addition of 1 M sodium acetate buffer (pH 5.0). The reaction was allowed to continue for 30 min at room temperature. The products were extracted three times with 20 ml ethyl ether and the ether evaporated to dryness under $N_2$. The residue was then partitioned between organic and aqueous (NaOH) phases as described above. Control tubes were processed in a similar manner without the addition of sodium borohydride.

*Binding of MENT metabolites to estrogen receptors.* Aromatized products were evaluated for their ability to competitively inhibit binding of [$^3$H]$E_2$ to estrogen receptors (ER) in rat uterine cytosol as follows. Female rats weighing 140–150 g were ovariectomized and killed 24 h later. The uteri were removed, weighed and minced with scissors. All subsequent steps were carried out at 4°C. The minced tissue was homogenized in 2 vol of 10 mM Tris–HCl buffer (pH 7.4) containing 1.5 mM EDTA, 0.5 mM DTT, 10 mM $Na_2MoO_4$, 10% glycerol and 1 mM phenyl methyl sulfonyl fluoride. The homogenate was centrifuged at 1000 *g* for 30 min. The supernatant was recentrifuged at 105,000 *g* for 90 min and the final supernatant (uterine cytosol) used for competitive binding studies.

Unlabeled MENT (3.5 nmol per tube) was incubated with placental microsomes (prepared and stored without cold androstenedione) for 60 min in the presence of the NADPH generating system. Incubations were also carried out in the absence of NADPH or in the presence of 10 $\mu$mol of R76713. The medium was extracted 3 times with 5 ml ethyl ether and the ether evaporated. The residue was dissolved in 1.0 ml assay buffer for receptor binding studies. Duplicate aliquots (50 $\mu$l) were incubated with 200 $\mu$l of uterine cytosol (300 $\mu$g protein) and [$^3$H]$E_2$, in a total volume of 0.4 ml for 18 h at 4°C. Following incubation, 0.5 ml of a suspension containing 1.25% charcoal (Norit A) and 0.125% dextran T-70 in assay buffer was added. After 10 min at 4°C, the samples were centrifuged at 1000 *g* for 10 min and the radioactivity in the supernatant determined. The percentage of [$^3$H]$E_2$ bound to the receptors was calculated.

In another experiment, [$^3$H]MENT was incubated with placental microsomes and the radioactivity was partitioned between the organic and aqueous phases. The binding of the radioactivity from the two phases to ER was determined. In addition, competitive inhibition of aqueous phase metabolites by MENT, $E_2$ and $MeE_2$ was investigated.

## RESULTS

The presence of aromatase activity in the placental microsomal preparation was confirmed by measuring the formation of tritiated water from [1$\beta$ $^3$H]androstenedione (data not shown). A linear relationship was observed between protein concentration and duration of incubation and radioactivity released into the aqueous phase.

*Metabolism of [$^3$H]T and [$^3$H]MENT by placental microsomes*

*TLC of reaction products.* $^3$H-androgens were incubated with microsomes and the ether extracts were fractionated by TLC using methylene chloride–ethyl acetate (8:2, v/v) as mobile phase. Following incubation of [$^3$H]T and [$^3$H]MENT with microsomes, new peak areas of radioactivity corresponding to [$^3$H]$E_2$ and $MeE_2$ standards, respectively, appeared (Figs 1 and 2). The $R_f$ value for the new radioactive product seen after incubation of [$^3$H]MENT with the microsomes was similar to that of $MeE_2$ visualized under UV light. When incubation of the androgens was carried out in the presence of R76713, the aromatase inhibitor, no radioactivity corresponding to the estrogens was present.

*Solvent partitioning of metabolites.* To further characterize the incubation products, extracts were partitioned between an organic phase and NaOH. The results presented in Table 1 show the distribution of radioactivity in the two phases. When [$^3$H]T or [$^3$H]MENT was incubated with placental microsomes in the presence of NADPH, there was a 20-fold increase in the radioactivity extractable into the base (0.4 N NaOH). This indicates the conversion of the androgens to a phenolic steroid. Furthermore, if the incubation was carried out in the presence of R76713, there was a marked reduction in androgen metabolism indicating that an active aromatase is required to convert the androgens to products that are extractable into NaOH. The metabolite(s) present in the NaOH phase were extracted with ethyl ether, concentrated and chromatographed on TLC plates using chloroform–toluene–methanol (14:7:2, by vol) as the mobile phase. The chromatogram showed a single radioactive spot which did not correspond with the MENT standard but whose mobility was identical to that of nonradioactive $MeE_2$ standard (Fig. 3). It should be noted that in the solvent system used here the mobility of MENT and its aromatized product is reversed compared to the results using a different solvent system shown in Fig. 2.

In order to rule out the possibility that exposure to strong base causes spontaneous aromatization of the intermediate metabolites of androgens, the residue from the ether extracts were treated with sodium borohydride before base extraction. The results (Table 2) show that the amount of radioactivity extracted into the base was not altered significantly due to the sodium borohydride treatment. Thus the base extractable radioactivity truly represents phenolic compounds resulting from enzymatic aromatization.

*Binding to ER.* In an attempt to determine whether estrogens were derived from the incubation of MENT

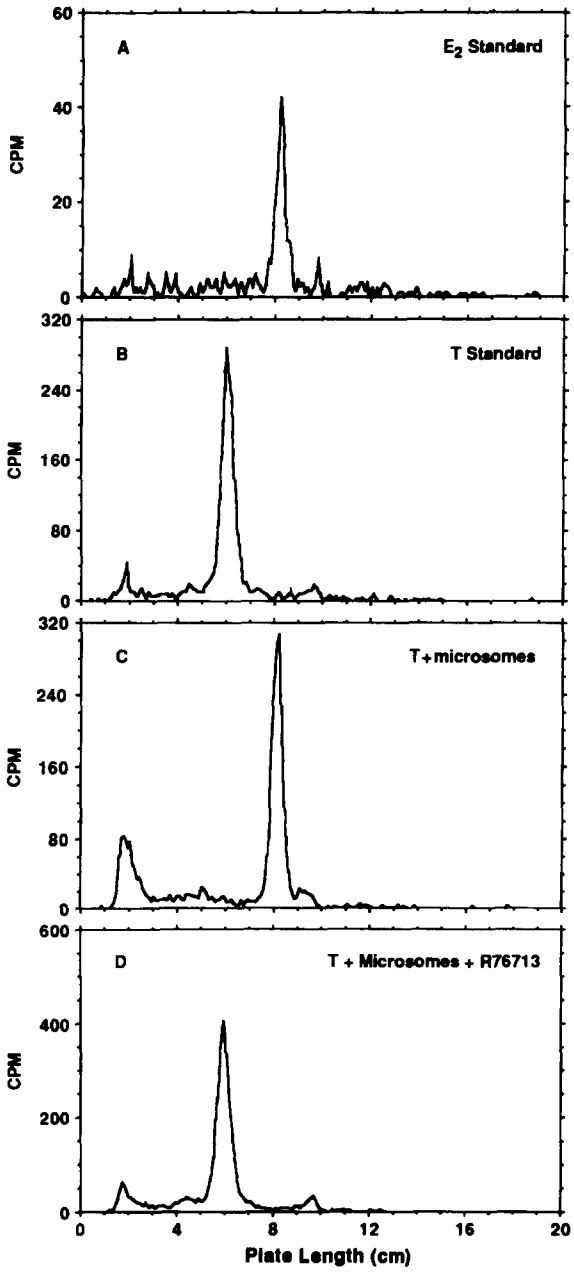


Fig. 1. Radiochromatogram of ether extract following incubation of [$^3$H]T with human placental microsomes. Microsomes and [$^3$H]T were incubated with NADPH for 60 min and the steroids extracted with ethyl ether. The ether was evaporated and the residue dissolved in acetone and spotted on to silica gel TLC plate and chromatographed using methylene chloride–ethyl acetate (8:2, v/v) as the mobile phase. Radioactivity on the plate was determined by scanning it with BIOSCAN. (A and B) Show the location of [$^3$H]$E_2$ and [$^3$H]T standards, respectively. (C) Shows the distribution of radioactivity following the incubation of [$^3$H]T with the microsomes. The mobility of the principal peak is similar to that of [$^3$H]$E_2$. (D) Shows the effect of R76713 (10 $\mu$mol) on [$^3$H]T aromatization.

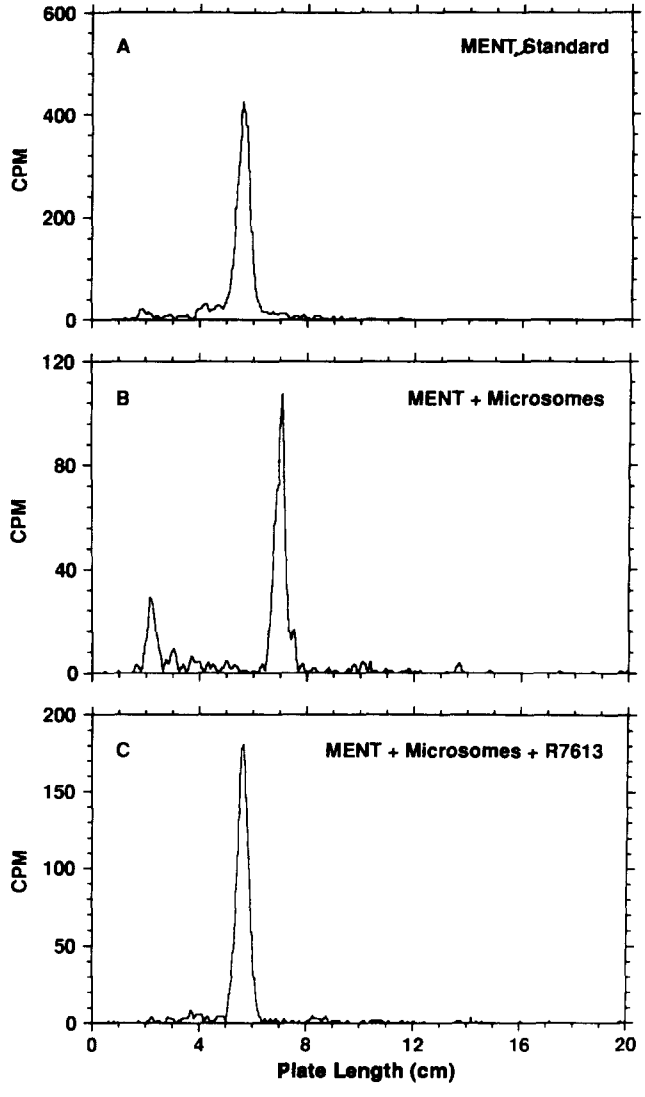


Fig. 2. Radiochromatogram of the ether extract following incubation of [$^3$H]MENT with human placental microsomes. See Fig. 1 for details. (A) Shows the location of [$^3$H]MENT standard. (B) Shows the distribution of radioactivity following incubation of [$^3$H]MENT with the aromatase system while (C) shows the effect of R76713.

with placental microsomes, binding of the incubation products to rat uterine ER was investigated. T showed no significant competitive binding to ER. MENT, on the other hand, showed low but significant binding to the ER (Fig. 4). However, $MeE_2$ was a very potent

*Table 1. Metabolism of [$^3$H]T and [$^3$H]MENT by placental microsomes (Mic)*

| | cpm in base ($\times 10^{-3}$) | cpm in BP ($\times 10^{-3}$) |
|---|---|---|
| 1. [$^3$H]T | 34 | 726 |
| 2. [$^3$H]T + Mic + NADPH | 809 | 126 |
| 3. [$^3$H]T + Mic − NADPH | 40 | 832 |
| 4. [$^3$H]T + Mic + NADPH + R76713 | 84 | 734 |
| 5. [$^3$H]MENT | 44 | 1027 |
| 6. [$^3$H]MENT + Mic + NADPH | 827 | 89 |
| 7. [$^3$H]MENT + Mic − NADPH | 36 | 728 |
| 8. [$^3$H]MENT + Mic + NADPH + R76713 | 55 | 711 |

Tritiated androgens (in duplicate) were incubated with placental microsomes with or without NADPH and with R76713 (10 $\mu$mol) for 60 min. The steroids were extracted and partitioned between 0.4 N NaOH (base) and BP and the total radioactivity in each fraction determined.

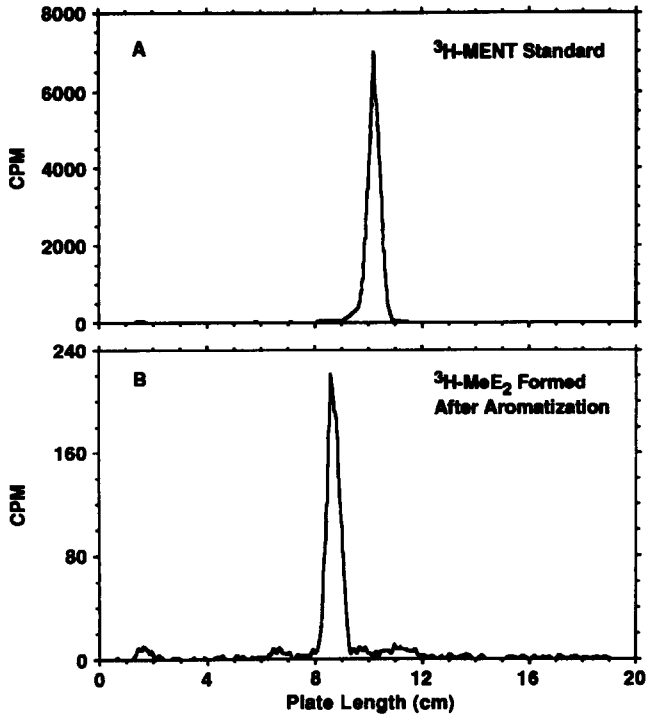


Fig. 3. Radiochromatogram of the base extract (0.4 N NaOH) following incubation of [$^3$H]MENT with placental microsomes. Following incubation, the steroids were extracted and partitioned between BP and 0.4 N NaOH. The chloroform–toluene–methanol (14:7:2, by vol) solvent system was used as the mobile phase. The scan shows [$^3$H]MENT standard (A) and its aromatized derivative (B). The mobility of the aromatized derivative was similar to that of cold MeE$_2$ standard visualized under UV light.

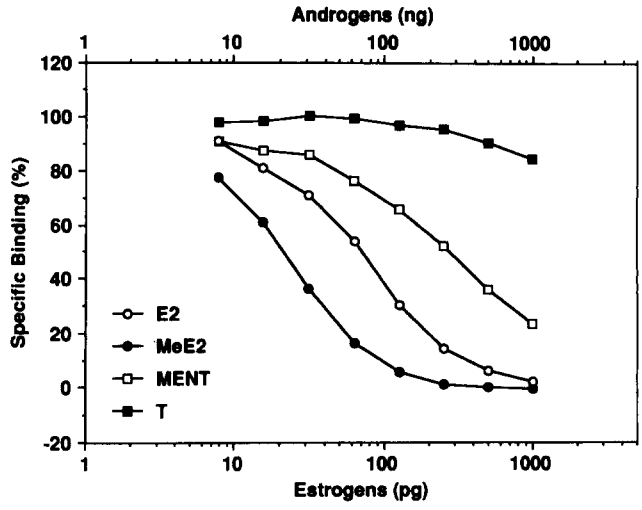


Fig. 4. Competitive inhibition of [$^3$H]E$_2$ binding to rat uterine ER by E$_2$, MeE$_2$, T and MENT. E$_2$ as well as MeE$_2$ show parallel displacement curves, with MeE$_2$ being 3 times more potent than E$_2$. MENT showed significantly low competition for the E$_2$ receptors. The ED$_{50}$ of MENT was approx. 10,000 fold higher than that of MeE$_2$. Note the different scales for estrogens and androgens.

competitor for ER. Furthermore, the competitive binding of MeE$_2$ to ER was greater than that of E$_2$. The ED$_{50}$ for the displacement of [$^3$H]E$_2$ by MeE$_2$, E$_2$ and MENT were approx. 20 pg, 70 pg and 250 ng, respectively.

Nonradioactive MENT (1 $\mu$g) was incubated with placental microsomes, the metabolites extracted with ethyl ether and used for the competitive binding studies. The reaction products in the extract showed complete displacement of [$^3$H]E$_2$ (Table 3). When incubation was carried out in the absence of NADPH or in the presence of R76713, there was decreased competition for the ER.

In another experiment, the radioactive product obtained after incubation of [$^3$H]MENT with placental microsomes was examined for binding to ER. The radiolabeled metabolite was found to bind to ER and could be displaced by the addition of MeE$_2$ or E$_2$ but not by MENT (Table 4).

*Table 2. Distribution of radioactivity after sodium borohydride treatment of incubation products*

| | cpm in base ($\times 10^{-3}$)[a] | cpm in BP ($\times 10^{-3}$)[a] |
|---|---|---|
| 1. [$^3$H]T | 671 | 151 |
| 2. [$^3$H]T (sodium borohydride) | 704 | 122 |
| 3. [$^3$H]MENT | 509 | 101 |
| 4. [$^3$H]MENT (sodium borohydride) | 416 | 110 |

Tritiated androgens (1 $\mu$Ci each) were incubated with placental microsomes and NADPH for 60 min. The metabolites were extracted with ether and dried under N$_2$. Half the tubes were treated with sodium borohydride. All the samples were extracted with ether, dried under N$_2$ and partitioned between base and BP. Sodium borohydride reduces the A-ring of metabolites of 19-norprogestins and prevents their chemical aromatization by strong acid or base. Results show that there is no significant difference in the distribution of radioactivity in base with and without sodium borohydride treatment. This suggests that the androgens were already aromatized before exposure to the base.

[a]Mean from two experiments.

## DISCUSSION

The results of the present study confirm that the microsomal fraction of human placenta contains an active aromatase system capable of converting T to E$_2$. The same placental microsome preparation that metabolized T to E$_2$ also converted the synthetic androgen, MENT, to a phenolic product that migrates with

*Table 3. Inhibition of [$^3$H]E$_2$ binding to rat uterine ER by MENT following incubation with placental microsomes*

| Incubation | [$^3$H]E$_2$ binding (% inhibition) |
|---|---|
| 1. MENT + NADPH | 97 |
| 2. MENT − NADPH | 32 |
| 3. MENT + NADPH + R76713 | 66 |

MENT (1 $\mu$g) (in duplicate) was incubated with placental microsomes with and without cofactors and R76713 (10 $\mu$mol). Incubation mixtures were extracted with ether and dried under N$_2$. The residues were dissolved in 1 ml of assay buffer and aliquots of 50 $\mu$l were used in the binding studies.

*Table 4. Binding of radioactive metabolites to ER following incubation of [$^3$H]MENT with placental microsomes*

| Source | cpm bound (%) |
|---|---|
| 1. Base fraction (0.4 N NaOH) | 29.2 |
| 2. Base fraction + MENT (2 ng) | 22.5 |
| 3. Base fraction + $E_2$ (2 ng) | 9.0 |
| 4. Base fraction + $MeE_2$ (2 ng) | 4.3 |
| 5. BP fraction | 2.5 |

[$^3$H]MENT (1 $\mu$Ci) was incubated with placental microsomes for 60 min and the radioactivity partitioned between 0.4 N NaOH and BP. Duplicate aliquots (18,000 cpm) from each fraction were incubated with rat uterine ER and percent of added radioactivity bound to the receptors determined after separation of the bound and free fractions by charcoal treatment.

$MeE_2$, the most likely estrogenic metabolite of MENT, in two TLC systems and that binds to rat uterine ER. Furthermore, the production of this MENT metabolite was inhibited by an aromatase inhibitor. In addition, selective extractability of radioactivity in base following incubation of [$^3$H]MENT with aromatase suggests the presence of true phenolic compounds. Earlier work by Moslemi *et al.* [18] compared the effects of MENT on aromatization of [1$\beta$-$^3$H]androstenedione by human and equine placental microsomes. MENT competitively inhibited the aromatization of androstenedione by human ($K_m$ = 5 nM) and equine ($K_m$ = 2 nM) placenta. More recently, Moslemi *et al.* [19] reported that unlike equine placental microsomes, human placental microsomes did not aromatize MENT. The reason for the diverse findings is not clear at this time.

Some structural modifications of the steroid nucleus such as the presence of 1$\alpha$ or 2$\alpha$ methyl group on 17$\beta$-hydroxy-4-androsten-3-one are known to inhibit aromatization. The 5$\alpha$-reduced metabolites of T and androstenedione and 6$\alpha$-fluorotestosterone have been shown to be nonaromatizable steroids [20]. Aromatization of 19-norandrogens was shown to be slower and less efficient compared to C-19-methyl steroids [21, 22]. However, recent *in vitro* studies using microsomal preparations from equine testis [23, 24] and porcine Leydig cells have shown equal rates of aromatization of androgens and norandrogens. It has also been shown that a single estrogen synthetase is present in equine testicular microsomes which catalyzes the aromatization of both C-19 methyl and 19-norandrogens. In addition it was suggested [25] that a single hydroxylation at C-1 would be sufficient to aromatize norandrogens.

Based on the chemical structure, the presence of a 7$\alpha$-substitution group on 19-nortestosterone was thought to be inhibitory to its aromatization since a number of 7$\alpha$-substituted androgen derivatives were found to have aromatase inhibitory activity [26, 27]. The methyl substitution is not inhibitory since 17$\alpha$-methyl testosterone is a good substrate for the aromatase [21].

From the literature (see review) [21] it can be inferred that 19-nor-steroids are aromatized either not at all or less efficiently than T. For example, whether or not aromatization of norethindrone (NET) takes place has been controversial. In humans, it was reported that as much as 1.0% of an orally administered dose of NET appeared in the phenolic fraction of the urine as ethynylestradiol [28, 29]. However, later it was shown to be an artifact of the method used [30, 31]. In human placental tissues, 19-norsteroids such as norgestrel [16], 19-norandrostenedione [16, 30] and norethindrone [17] are metabolized to 1$\beta$-hydroxylated derivatives. Exposure of these 1$\beta$-hydroxylated compounds to acid or base results in nonenzymatic, chemical aromatization to the corresponding estrogens [16, 30]. However, 1$\beta$-hydroxylated 19-norsteroids will not undergo chemical aromatization when treated with sodium borohydride [30]. Sodium borohydride treatment of 1$\beta$-hydroxylated 19-norsteroids results in the reduction of the 4-en-3-one grouping of the A ring, thereby preventing chemical aromatization by acid or base. In the present study, the treatment with sodium borohydride did not result in a significant decrease in the radioactivity extracted with the base. Hence it can be argued that aromatization of MENT was enzyme catalyzed. Furthermore, the conversion of MENT to phenolic compounds could be blocked by a specific aromatase inhibitor.

For use as a male contraceptive or for replacement therapy, an androgen of choice should be able to maintain all the beneficial actions of T while avoiding some of its undesirable effects. In our previous studies [8, 9] we have shown that MENT does not undergo 5$\alpha$-reduction *in vitro* or *in vivo* thus avoiding hyperstimulation of the prostate while maintaining other general physiologic actions of testosterone. The present studies are consistent with aromatization of MENT. MENT has been shown to maintain sexual behavior in rabbits [32] and rats [33]. Whether or not the above actions are mediated by the aromatized product of MENT is not known. The induction and/or maintenance of male sexual behavior by testosterone depends in part upon aromatization of testosterone to estradiol [10, 34]. Nonaromatizable androgens such as DHT were relatively ineffective in inducing male sexual behavior [35]. Aromatase enzyme activity has been shown to be present in the brain areas associated with reproductive functions [36]. A role for estrogens formed by hypothalamic and limbic aromatase enzyme has also been implicated in the feedback regulation of gonadotropin secretion in men and nonhuman primates [11, 37, 38] but not in rodents [39, 40].

Although the phenolic endproduct of MENT aromatization was not structurally characterized, it exhibited specific binding to rat uterine ER. The generation of this receptor binding metabolite was inhibited by R76713, an aromatase inhibitor. This inhibitor has been shown to be very specific towards the aromatase enzyme both *in vitro* and *in vivo* [41]. Even though we were able to procure a small amount of authentic

$MeE_2$, it was not sufficient to perform recrystallization studies. The characterization of the aromatized product of MENT by other methods such as GCMS and recrystallization will be undertaken when a sufficient quantity of pure $MeE_2$ is synthesized.

These observations demonstrate that MENT undergoes aromatization and that the endproduct obtained behaves like an estrogen. The ability of MENT to undergo aromatization might be of biological significance when it is used as a replacement androgen.

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