Prostate-Sparing Effects in Primates of the Potent Androgen 7α-Methyl-19-Nortestosterone: A Potential Alternative to Testosterone for Androgen Replacement and Male Contraception*

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ABSTRACT

7α-Methyl-19-nortestosterone (MENT) is a potent synthetic androgen that cannot be converted to dihydrotestosterone. In this study we determined the relative androgenic, antigonadotropic, and anabolic potencies of testosterone vs. MENT in the nonhuman primate Macaca fascicularis. In castrated monkeys, dose-response relationships were generated for the effects of testosterone and MENT on gonadotropin levels, prostate growth, body weight, and lipid metabolism. In a pilot study, four monkeys were castrated, and magnetic resonance imaging (MRI) was used to document a 50% loss of prostate volume within 8 weeks, verifying that MRI is a reliable means to measure prostate size in this species. Two additional groups of six monkeys each were then castrated and serially administered four graded dosages of testosterone or MENT via osmotic minipumps over 20 weeks. Complete suppression of LH was achieved with a minimum of 0.3 mg/day MENT, compared to 3.0 mg/day testosterone. MENT supported body weight 10 times more potently than did testosterone. Baseline prostate volumes were maintained with 0.1–0.2 mg/day MENT vs. 0.3 mg/day testosterone. Thus, in monkeys, MENT is 10 times more potent than testosterone with regard to the clinically desirable endpoints of gonadotropin suppression and anabolism, but only twice as potent at stimulating prostate growth. These results suggest that MENT may have a wider therapeutic index than testosterone for human androgen replacement and male contraception. (J Clin Endocrinol Metab 83: 4212–4219, 1998)

TESTOSTERONE is metabolized in vivo into two principal active hormones (1). Approximately 6–8% is converted to dihydrotestosterone (DHT) by 5α-reductase, which is most abundant in male accessory sex organs and sexual skin (2, 3). Approximately 0.3% is aromatized to estradiol, primarily in brain, liver, and adipose tissue (4, 5). The sum of the actions of testosterone, DHT, and estradiol determine the overall biological impact of testosterone. Together, these hormones produce a host of physiological actions that can be described as androgenic (growth of accessory sex organs, genital virilization, potency), anabolic (maintenance of muscle, kidney, salivary gland, and liver growth and function), antigonadotropic (suppression of LH and FSH secretion), and behavioral [libido, central nervous system (CNS) gender imprinting].

In adult men, testosterone itself is thought to be the principal hormone governing libido, potency, gonadotropin feedback regulation, and growth and function of nongenital tissues, such as muscle, kidney, liver, and bone (6–10). Estradiol influences CNS gender imprinting, sexual behavior, and gonadotropin regulation and exerts beneficial effects on serum lipids (11–19). In general, the biological actions of both testosterone and estradiol are desirable in men. Conversely, DHT has no known unique beneficial function in adult men, but mediates several untoward actions, including prostate hypertrophy, balding, and acne (20). Thus, the ideal steroid for therapeutic androgen replacement would be a potent testosterone agonist that does not undergo 5α-reduction to DHT but can be aromatized to an estrogen. In theory, 7α-methyl-19-nortestosterone (MENT) should be such a compound (21, 22). This synthetic androgen is more potent in rodents than testosterone, but resists 5α-reduction. Consequently, it should accomplish the beneficial physiological effects of testosterone and estradiol without the deleterious side-effects of DHT.

In the present study, we sought to determine whether such favorable characteristics of MENT action are indeed manifest in the nonhuman primate Macaca fascicularis. The reproductive systems of male monkeys and humans are similar in many ways (23). Specifically, in both species, prostate anatomy is homologous (24, 25), the prostate is very sensitive to androgen stimulation and withdrawal (25–29), DHT is the
dominant intraprostatic androgen (30), and the 5α-reductase isoenzymes are structurally and functionally comparable (31). Thus, monkeys are a reasonable model from which to draw probable conclusions about human physiology with regard to MENT and testosterone actions.

**Materials and Methods**

**Monkeys**

Sixteen adult male monkeys of the species *M. fascicularis* were obtained from the University of Washington Regional Primate Research Center (Seattle, WA; four castrate controls and six each to receive testosterone or MENT). At the start of the study their ages ranged from 4–7 yr (castrate controls, 4.8 ± 1.5; testosterone group, 5.1 ± 1.0; MENT group, 5.4 ± 0.8). Body weights ranged from 3.8–7.3 kg (castrate controls, 5.5 ± 1.4; testosterone group, 4.8 ± 0.6; MENT group, 5.6 ± 1.1). There were no significant differences among the three groups with respect to initial ages or body weights. All animals were housed at a constant temperature (23 ± 2°C) in cages concealed from outside light and were maintained on a 12-h light, 12-h dark cycle (lights on at 0600 h). They were anesthetized with im ketamine injections for all procedures except castrations (bilateral orchidectomies), which were performed under general anesthesia using inhaled fluothane. Blood samples were obtained via the osmotic pumps implanted subdermally in the back (Alza Pharmaceutics, Palo Alto, CA). Each androgen was administered in 45% (wt/vol) solution of 2-hydroxypropyl-β-cyclodextrin (Cyclodextrin Technologies Development, Gainesville, FL). Steroid concentrations varied from 5–25 mg/mL for testosterone and from 0.5–4.2 mg/mL for MENT, depending on the dosage being delivered.

**Experimental design**

In the first stage of the experiment 4 animals designated castrate controls were castrated, and prostate volumes were subsequently assessed by magnetic resonance imaging (MRI) at biweekly intervals for 8 weeks. Serial 5-mL blood samples were drawn to determine serum testosterone and bioactive LH levels before and after castration. Six such samples were obtained over a 10-day period before castration, and 18 samples were obtained over a 9-week period after castration. In the second stage of the experiment 2 groups of 6 monkeys each were used to establish dose-response relationships for the androgenic, antigonadotropic, and anabolic activities of testosterone vs. MENT. After a 14-day observation period, monkeys were castrated and simultaneously begun on continuous infusions of either testosterone acetate or MENT, delivered via Alzet osmotic pumps implanted subdermally in the back (Alza Pharmaceuticals, Palo Alto, CA). Each androgen was administered in 4 consecutive, decreasing dosages for 4 weeks each (testosterone group, 5.0, 3.0, 1.0, and 0.3 mg/day; MENT group, 1.0, 0.3, 0.1, and 0.03, mg/day). All animals were monitored for 4 additional weeks after the last treatment dose. MRI of the prostate was performed before castration and at the end of each treatment period. For steroid and gonadotropin levels, 5 blood samples (5 mL each) were drawn over a 14-day period before castration, then once at the end of the second week of each treatment period, daily for 3 days at the end of the third and fourth weeks of each treatment period, and finally 3 and 4 weeks after the last treatment. To deliver the appropriate daily androgen doses, osmotic pumps were changed weekly during the first treatment period, biweekly during the second period, and every 4 weeks during the last 2 periods.

**Prostate volumes**

Prostate volumes were determined using MRI. A General Electric Sigma 1.5-Tesla MRI System was employed, with a G.E. Independent Console for image analysis (Milwaukee, WI). Contiguous 3.0-mm thick axial slices with 0.3-mm spacing were obtained through the pelvic region extending from above the seminal vesicles to below the prostate. Depending on its size, the entire prostate could be visualized in five to seven slices. Computer displays of prostate images from each slice were manually outlined, and the surface area contained within this region of interest was measured using G.E. System 5.4 software. Prostate volume in cubic millimeters was then calculated with the following formula: mean prostate surface area/slice (mm²) × number of slices × 3.5 mm²/slice. During the same procedure an estimate of seminal vesicle volume was also obtained.

**Hormone and lipid assays**

Blood samples used in hormone assays were permitted to clot at room temperature for 1 h, then were centrifuged at 1500 × g for 20 min. Serum was separated and stored immediately at −20°C for future study. Plasma was obtained for lipid determinations and stored immediately at −70°C. Serum testosterone levels were measured by RIA with methods previously described (33), using reagents from the WHO Matched Reagent Program. The assay sensitivity was 0.17 nmol/L; the inter- and intraassay coefficients of variation were 8.1% and 4.1%, respectively. Serum MENT levels were assessed with a specific RIA as previously described (34). The lower limit of detectability was 28 pg/mL. Testosterone and DHT cross-react only minimally in this assay. Serum bioactive LH was measured using the method of Dufau (35). This assay quantitates the LH-dependent secretion of testosterone from dispersed mouse Leydig cells in primary culture and has been shown to be suitable for monkey blood. The assay sensitivity was 2.4 mIU/mL. Hormone assay sensitivities were determined by the first point discernible from zero on standard curves. Lipid analyses were performed at the Northwest Lipid Research Laboratories, University of Washington (Seattle, WA). Total plasma cholesterol levels were assessed enzymatically (36) on an Abbott Spectrum multichromatic analyzer (Abbott Laboratories, North Chicago, IL) using a procedure standardized to the Center for Disease Control’s reference method. The cholesterol concentration in high density lipoprotein (HDL) was measured enzymatically after precipitation with dextran sulfate-magnesium (37), whereas cholesterol in the HDL₂ and HDL₃ subfractions was assayed after differential precipitation (38). Lipoprotein(a) was measured with a double monoclonal antibody-based enzyme-linked immunosorbent assay as previously described (39).

**Results**

The first stage of the experiment was designed to determine the time course of changes in gonadotropins, testosterone, and prostate volume after castration in *M. fascicularis*. This pilot study was performed to assure that the duration of planned steroid infusions was adequate to assess responses in the above parameters as well as to confirm that prostate volumes could be assessed using MRI. MRI proved to be a precise modality for measuring prostate size in monkeys, confirming the one prior report on this topic (40). Prostate volume decreased by approximately 50% within 8 weeks after castration (Fig. 1A). As expected, serum testosterone levels fell dramatically within 1 day after castration and remained at least 20 times lower than precastrate levels thereafter (Fig. 1B). In the absence of testosterone-mediated negative feedback, serum bioactive LH levels rose sharply within 1 day after castration (Fig. 1C).

The second stage of the experiment assessed the relative potencies of testosterone vs. MENT in castrated adult monkeys (six per group) for gonadotropin suppression, stimulation of prostate growth, regulation of body weight, and alteration of serum lipids. As shown in Fig. 2, serum levels of testosterone and MENT were proportional to the graded doses of each androgen, indicating appropriate drug delivery via the osmotic pumps.

Both androgens showed steep dose-response curves for
gonadotropin suppression (Fig. 3). Similar phenomena have been reported in male rats (41). The minimal testosterone dose required to achieve complete LH suppression was 3.0 mg/day, compared with only 0.3 mg/day of MENT. In other words, MENT was 10 times more potent than testosterone with regard to gonadotropin suppression in castrated monkeys.

The 0.3 mg/day dose of testosterone failed to suppress LH (Fig. 3) even though it restored normal serum testosterone levels (Fig. 2). It is a common clinical observation that adequate testosterone replacement does not suppress LH to normal in either castrated monkeys (42) or humans with primary testicular failure (43). The reason for this is unclear, but makes the measurement of LH a relatively useless indicator of the adequacy of testosterone replacement (in contrast to the usefulness of TSH measurements in thyroid replacement). It is possible, but not proven, that other testicular products (such as estradiol, DHT, or inhibin B) have partial inhibitory effects on LH secretion.

Both androgens supported prostate growth in a dose-dependent manner, each stimulating the gland to supranormal size at higher doses (Fig. 4). The dose of testosterone required to maintain normal prostate volume was 0.3 mg/day, compared with 0.1 mg/day or slightly higher for MENT. Thus, MENT was only 2–3 times more potent than testosterone in terms of stimulating prostate growth, in contrast to its much greater differential potency for gonadotropin suppression.
Seminal vesicle volumes were affected by both androgens in a manner parallel to that of prostate volumes (data not shown).

The relative anabolic potencies of testosterone vs. MENT were roughly estimated by measuring total body weight. As shown in Fig. 5, both androgens increased body weight to above baseline; however, MENT was 10 times more potent than testosterone in this respect. Although body weight is an indirect measure of anabolism, it has been shown previously that androgen-mediated increases in body weight arise principally from anabolic effects on muscle, liver, and kidney tissue (20).

The effects of supplemental testosterone and MENT on serum lipids are shown in Fig. 6. Untreated castrates and those replaced with low doses of either testosterone or MENT had elevated total cholesterol, HDL, and HDL₂ levels, whereas higher doses of both androgens suppressed these values. Neither steroid affected HDL₃ or lipoprotein(a) significantly. Overall, MENT was approximately 10 times more potent than testosterone in its effects on lipids.

If MENT and testosterone actions are compared relative to the mass of each androgen in blood, the difference in bio-potencies appears even greater than when the administered doses are compared. The minimal blood level required to achieve complete LH suppression was 99 nmol/L for testosterone vs. 1.6 nmol/L for MENT. This suggests that MENT may actually be more than 60 times more biopotent than testosterone with regard to this end point, rather than 10 times, as would be calculated from the administered doses. Similar findings have been reported in castrated rats (41). In these animals, although MENT was about 10 times more potent than testosterone at suppressing gonadotropins and supporting muscle weight based on administered doses, it was at least 30 times more potent based on relative mass in blood.

Discussion

This study establishes dose-response relationships in non-human primates for testosterone and MENT effects on LH regulation, prostate size, body weight, and lipid metabolism. The dose-response curves are adequate because the regimens chosen for both steroids ranged from subphysiological to supraphysiological with regard to the principal end points. Our results demonstrate that in monkeys MENT has 10 times more antigonadotropic and anabolic potency than testosterone, but is only 2–3 times more potent at stimulating prostate growth. These findings are very similar to those previously reported in rats. The potential clinical significance of these findings is currently being investigated in monkeys.
reported for rodents. In castrated rats MENT is 12 times more potent than testosterone at suppressing the postcastration rise in gonadotropins and 10 times more potent at maintaining muscle mass, but only 4 times as potent at stimulating growth of the ventral prostate and seminal vesicles (41). Similarly, in castrated mice MENT is only 3 times as potent at maintaining seminal vesicle mass (44).

MENT is believed to be more biopotent overall than testosterone both because 19-nor-testosterone derivatives in general demonstrate this property (10) and also because the 7α-methyl group in MENT greatly enhances its binding affinity for the androgen receptor and increases subsequent nuclear retention (45). The blunted action of MENT on male accessory sex glands relative to its action on muscle is probably due to its resistance to enzymatic conversion to a 5α-dihydrosteroid (46). Testosterone is converted to DHT by 5α-reductase, which is especially abundant in accessory sex glands (2). DHT is 3–5 times more active than testosterone, probably because of its greater affinity for the androgen receptor (45). Hence, the action of testosterone is amplified in male reproductive organs, which explains its potent capacity to stimulate prostate and seminal vesicle growth. In contrast, MENT has been shown to resist 5α-reduction in rat prostate and epididymis despite an abundance of 5α-reductase activity in these tissues (47). Presumably, the 7α-methyl group sterically hinders 5α-reductase. Consequently, MENT’s actions in the male reproductive tract are not amplified as are those of testosterone. MENT is thus relatively less potent at stimulating the growth of male accessory sex glands compared with its ability to suppress gonadotropins and maintain muscle mass. The hypothesis that testosterone is relatively more potent at stimulating prostate and seminal vesicle growth because it is converted in those tissues to DHT has been confirmed empirically in rats (41). 5α-Reductase inhibitors blunt the effects of testosterone on reproductive tissues, but have no impact on testosterone action in muscle.

**Fig. 5.** Body weight in monkeys receiving testosterone (light shading) or MENT (dark shading). Administered doses of each androgen are indicated in milligrams per day on the abscissa. Body weight is expressed as a percentage of the baseline relative to values in intact animals before initiation of drug therapy. Actual baseline body weights were 4.8 ± 0.6 kg for the testosterone group and 5.6 ± 1.1 kg for the MENT group. Values are the mean ± SEM (n = 6/group).

**Fig. 6.** Serum lipids in monkeys receiving graded doses of testosterone (open circles) or MENT (closed circles). Values on the abscissa represent the administered dose in milligrams per day of testosterone or MENT (in that order). Intact and Castrate refer to animals receiving no exogenous androgens. Values are the mean ± SEM (n = 6/group).
which lacks 5α-reductase activity (3, 6, 48, 49), or any effect on the actions of MENT in any tissue. The fact that 5α-reductase inhibitors do not modify the action of MENT on male reproductive tissues is consistent with its lack of 5α-reduction in those tissues.

All hormonal male contraceptive prototypes include supplemental androgens, which are used either to suppress gonadotropin secretion (and thus spermatogenesis) or to replace endogenous androgens that have been eliminated with inhibitory GnRH analogues or progestins (50). The ideal androgen for this purpose would be more potent at suppressing gonadotropins than at stimulating prostate growth, so that a dose could be used that would accomplish the former outcome but not the latter. In castrated rats, a dose of MENT exists (9 μg/day) that suppresses gonadotropin secretion and maintains normal muscle mass, but does not support prostate and seminal vesicle weight (41). In contrast, the minimal dose of testosterone that suppresses gonadotropins and maintains muscle mass (90 μg/day) also sustains normal prostate and seminal vesicle weight. In castrated monkeys, we found that the minimal MENT dose required to suppress LH (0.3 mg/day) maintained approximately normal prostate size. In contrast, the minimal testosterone dose required to suppress LH (3.0 mg/day) stimulated prostate growth to twice normal size. Thus, in both rats and monkeys, MENT can be used to suppress gonadotropins completely without hyperstimulating the prostate. This is not the case for testosterone, which hyperstimulates the prostate in both species at the minimal dose required to suppress gonadotropins. Likewise, studies in normal men administered exogenous testosterone at contraceptive doses have documented mild prostatic enlargement (51, 52). Although these changes were not clinically manifest, the long term impact of such supra-physiological prostate stimulation is unknown. Clearly, MENT offers a wider therapeutic index than testosterone with regard to prostate hypertrophy and, in theory, with regard to stimulating the growth of incipient prostate cancer.

Theoretically, MENT action should also be blunted compared to that of testosterone in other tissues where 5α-reduction plays a significant role in androgen action, such as sexual skin. Thus, MENT should be relatively less likely to cause adverse effects such as androgenic alopecia and acne. Future studies are required to test this hypothesis.

It has been widely suspected that exogenous androgens used in hormonal male contraceptive regimens may raise intratesticular androgen concentrations sufficiently to stimulate spermatogenesis, attenuating the antifertility impact of the contraceptive (50, 53–55). The following lines of evidence suggest that 5α-reduced metabolites of testosterone actually mediate this undesirable effect. 1) DHT has been shown to be involved in the restoration of elongated spermatid maturation in testosterone-treated rats (56). 2) Men who become only oligospermic after prolonged testosterone treatment show increased production of 5α-reduced androgens compared with those who become azoospermic, implying that DHT formation may maintain low levels of spermatogenesis in these nonresponders (57). 3) Asian men, who are rendered azoospermic by androgen-based contraceptives far more reliably than are Caucasians (58), have significantly lower serum levels of 5α-reduced androgens (59). Thus, a non-5α-reducible androgen such as MENT offers an alternative to testosterone for male contraceptives that not only provides a superior side-effect profile, but may also enhance contraceptive efficacy.

MENT has, in fact, been tested as a component of a male contraceptive regimen in one small study (60). In this trial, rhesus monkeys were rendered profoundly hypogonadal with the GnRH agonist histerlin. After 8 months of treatment, MENT was added to the histerlin treatment and restored ejaculatory ability while maintaining azoospermia for 8 additional months. These findings demonstrate a promising role for MENT as a component of a hormonal male contraceptive.

It has been reported previously that testosterone, even at physiological levels, lowers serum HDL and HDL_2 in men (61–63), apparently by inducing hepatic triglyceride lipase activity (64). Consistent with these reports, we found that castrated monkeys and those replaced with low doses of either testosterone or MENT had elevated HDL and HDL_2 levels, whereas higher doses of both androgens suppressed these values. Overall, MENT showed the same 10-fold greater potency than testosterone with regard to its impact upon lipids as it showed for gonadotropin suppression and support of body weight. This suggests that 5α-reduction is not involved in testosterone regulation of lipid metabolism. Importantly, the minimum doses of both MENT and testosterone required to suppress LH and thus exert a contraceptive effect (0.3 and 3.0 mg/day, respectively) were associated with unaltered HDL and increased HDL_2.

Although it resists 5α-reduction, MENT does undergo aromatization to an estrogen (7α-methyl-estradiol) (65). This property may be important if MENT is to be used for androgen replacement, as estrogenic metabolites of testosterone have been implicated in gonadotropin regulation, CNS gender imprinting, and sexual behavior (11–15). Indeed, MENT has been shown to sustain male sexual behavior 5–20 times more potently than testosterone in castrated rats and hamsters (9, 66). Interestingly, in castrated mice a MENT dose sufficient to restore sexual behavior and seminal vesicle weight to the same extent as a larger dose of testosterone was comparatively less active at promoting aggressive behavior (44). Furthermore, aromatization of supplemental androgens reduces the degree to which these agents lower HDL levels, as this adverse consequence appears to be a pure androgen effect antagonized by estrogenic metabolites that suppress hepatic triglyceride lipase activity (16–18). As testosterone and MENT both undergo aromatization, it is not surprising that their relative potencies regarding lipid metabolism are similar to their relative potencies at all other target organs except those with high 5α-reductase activity.

In addition to their potential utility in hormonal male contraceptives, supplemental androgens are clinically useful in treating male hypogonadism, delayed puberty, certain hematological disorders, hereditary angioneurotic edema, and possibly various muscle-wasting conditions (43, 67). At present, testosterone esters (testosterone enanthate and cypionate) are the most commonly employed compounds (68). Unfortunately, these agents require frequent IM injections and are associated with wide fluctuations in plasma testosterone levels (69). To alleviate these problems, slow release
Illustration of this fact is the excellent safety profile of the beneficial role for 5α-reductase inhibitors. 

300–500 mg/day. This quantity could be delivered subdermally in currently available sustained release formulations that should last for at least 1 yr (21).

If MENT is to be used instead of testosterone for androgen replacement, an important question that must be addressed is whether there are any useful physiological functions mediated by 5α-dihydrosteroids that would not be subserved by an androgen that resists 5α-reduction. Although the actions of DHT are vital for differentiation of the male reproductive tract in the fetus (72), there is no known unique beneficial role for 5α-dihydrosteroids in adults. A powerful illustration of this fact is the excellent safety profile of the extensively studied 5α-reductase inhibitor, finasteride. Phase 3 trials involving hundreds of men studied for up to 3 yr revealed no major adverse effects of finasteride, except for slighter higher incidences of impotence (3.3% vs. 1.6%) and decreased libido (3.3% vs. 1.6%), problems that generally improved with continued treatment (73–75).

In summary, our results demonstrate that in primates, MENT is far more potent than testosterone with regard to the clinically desirable end points of gonadotropin suppression and anabolism, but has a relatively blunted capacity to support prostate growth. Prior studies in rats have shown that MENT is also far more potent at sustaining male sexual behavior and function. Thus, MENT can be delivered at doses that maintain the beneficial metabolic and behavioral effects of testosterone without hyperstimulating the prostate. Furthermore, as MENT is more biopotent than testosterone and is effective at low doses, it can be administered in more convenient formulations. MENT may therefore be superior to testosterone for androgen replacement in diverse clinical settings. Although a great deal of attention is currently focused upon the development and clinical use of tissue-selective estrogen receptor modulators such as raloxifene and tamoxifen (76), MENT is the first example of an androgen with relative tissue-selective actions.

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