Journal of Steroid Biochemistry & Molecular Biology 122 (2010) 212–218

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

The potent synthetic androgens, dimethandrolone (7α,11β-dimethyl-19-nortestosterone) and 11β-methyl-19-nortestosterone, do not require 5α-reduction to exert their maximal androgenic effects☆

Barbara J. Attardi*, Sheri A. Hild, Sailaja Koduri, Trung Pham, Laurent Pessaint, Jean Engbring, Bruce Till, David Gropp, Anne Semon, Jerry R. Reel

Division of Reproductive Endocrinology and Toxicology, BIOQUAL, Inc., 9600 Medical Center Dr., Rockville, MD 20850, United States

ARTICLE INFO

Article history:
Received 13 April 2010
Received in revised form 27 May 2010
Accepted 10 June 2010

Keywords:
Synthetic androgens
Hormonal therapy for men
5α-Reductase
Dutasteride
Accessory sex glands
Androgen receptor

ABSTRACT

Dimethandrolone (DMA: 7α,11β-dimethyl-19-nortestosterone) and 11β-methyl-19-nortestosterone (MNT) are potent androgens in development for hormonal therapy in men. As 5α-reduced androgens, such as 5α-dihydrotestosterone (DHT), may raise the risk of benign prostate hyperplasia, accelerate the development of prostate carcinoma, and increase male pattern baldness and acne, we investigated the role of 5α-reduction in the androgenic activity of DMA and MNT. The authentic 5α-reduced metabolites, 5α-dihydroDMA (5α-DHDMA) and 5α-dihydroMNT (5α-DHMNT), were prepared by chemical synthesis and compared *in vitro* and *in vivo* to the parent compounds. Both 5α-reduced androgens bound with high affinity to the rat androgen receptor (AR) and were potent inducers of transactivation of 3XHRE-LUC in CV-1 cells cotransfected with a human AR expression plasmid. To examine *in vivo* androgenic (stimulation of ventral prostate [VP] and seminal vesicle [SV] weights) and anabolic (stimulation of levator ani [LA] muscle weights) activity, 22-day-old castrate male rats were treated sc for 7 days with various doses of DMA, 5α-DHDMA, or testosterone (T) or MNT, 5α-DHMNT, or T and necropsied on day 8. 5α-DHDMA was at least threefold more potent than T in stimulating growth of the VP but only 30–40% as potent as DMA. 5α-DHMNT was four- to eightfold more potent than T, whereas MNT was approximately equipotent to T. To assess the possible role of 5α-reduction in VP and SV growth, castrate immature rats were treated with maximally effective doses of T, DHT, DMA, MNT, or the related 19-norandrogen, 7α-methyl-19-nortestosterone (MENT), or vehicle, with or without dutasteride (DUT), an inhibitor of 5α-reductases types 1 and 2. In rats treated with T + DUT, serum T was significantly higher ($P < 0.05$) than in rats treated with T alone, and serum DHT was decreased ($P < 0.001$) to levels observed in castrate vehicle-treated rats. DUT significantly reduced both VP and SV weights in T-treated rats, whereas there was no significant effect of DUT on weights of these accessory sex glands in rats treated with DMA, MNT, DHT, or MENT. These results indicate that inhibition of 5α-reductase activity *in vivo* does not affect the androgenic potency of DMA, MNT, or MENT.

1. Introduction

Testosterone (T) is the principal androgen secreted by Leydig cells. It exerts both androgenic effects involving growth stimulation and functional maintenance of the male reproductive tract and anabolic effects involving growth stimulation of nonreproductive organs such as muscle, kidney, liver, and submaxillary salivary glands [1]. T levels may decline in aging men resulting in clinical symptoms similar to those observed in hypogonadal men [2,3]. Current androgen replacement therapies involving the use of T have been shown to alleviate symptoms. When natural T is administered orally, however, only a small amount reaches the circulation due to absorption from the gastrointestinal tract into the portal blood and degradation by the liver (first-pass effect) [2,3]. The biological actions of T depend in part on its metabolism. In the male accessory sex organs and skin, T is converted by 5α-reductases to 5α-dihydrotestosterone (DHT), whereas in brain, adipose tissue, and gonads, T is converted to estradiol by aromatase cytochrome P450 [4].

Alternative androgens are being developed for hormonal therapy which may demonstrate greater potency, duration of action, and oral efficacy than T. Dimethandrolone (DMA: 7α,11β-dimethyl-19-nortestosterone) and the 11β-monomethylated

☆ A preliminary version of this work was presented at the 89th Annual Meeting of the Endocrine Society, June 2007.

* Corresponding author at: Molecular Endocrinology Laboratory, BIOQUAL, Inc., 9600 Medical Center Drive, Rockville, MD 20850, United States.
Tel.: +1 301 251 0633; fax: +1 301 251 1260.
E-mail address: bjattardi@bioqual.com (B.J. Attardi).

0960-0760/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2010.06.009

analogue of DMA (11β-methyl-19-nortestosterone, MNT) are potent orally active synthetic 19-norandrogens. We are currently assessing the 17β-undecanoic acid ester of DMA (DMAU) and the 17β-dodecylcarbonate ester of MNT (MNTDC) as potential agents for androgen therapy [5]. Cleavage of the 17β-ester bond in the circulation liberates the biologically active androgens. As a result of their potency and oral bioavailability, DMAU/DMA and MNTDC/MNT have the potential advantage that they could be used at relatively low doses.

5α-Reduced androgens such as DHT may raise the risk of benign prostate hyperplasia (BPH), accelerate the development of prostate carcinoma, and increase male pattern baldness and acne. Therefore, it was desirable to investigate whether DMA and MNT are 5α-reduced *in vivo,* potentially resulting in adverse effects similar to those of DHT. Authentic 5α-dihydroDMA (5α-DHDMA) and 5α-dihydroMNT (5α-DHMNT) were prepared by chemical synthesis and their activities compared *in vitro* (androgen receptor [AR] binding and transactivation in CV-1 cells mediated by AR) and *in vivo* (stimulation of ventral prostate [VP], seminal vesicles [SV], and levator ani [LA] muscle weights) with those of the parent compounds. The potential 5α-reduction *in vivo* of DMA and MNT was assessed indirectly as there are currently no assays to measure 5α-DHDMA or 5α-DHMNT in serum. Rats were treated with maximal stimulatory doses of various androgens in the absence or presence of the dual 5α-reductase inhibitor, dutasteride (DUT), at a dose which completely blocked conversion of T to DHT, but did not cause overt signs of toxicity. The effect of these treatments on androgenic endpoints was evaluated. The related 19-norandrogen, 7α-methyl-19-nortestosterone (MENT) was also tested as MENT had been shown previously not to undergo 5α-reduction *in vivo* or by rat liver, prostate, and epididymis *in vitro* [6,7]. The results of these experiments indicated that DUT treatment, which completely inhibited the conversion of T to DHT and significantly decreased androgenic effects in T-treated animals, did not alter the stimulatory effect of DMA, MNT, or MENT on growth of the VP and SV. Thus, inhibition of 5α-reductase activity *in vivo* does not affect the androgenic potency of DMA, MNT, or MENT.

2. Materials and methods

2.1. Chemicals

T and DHT were purchased from Steraloids (Newport, RI). DMA, 5α-DHDMA, MNT, 5α-DHMNT, MENT, and 5α-dihydroMENT (5α-DHMENT) were synthesized by the Southwest Foundation for Biomedical Research (San Antonio, TX) under contract NO1-HD-6-3255 and were >98–100% pure by HPLC and NMR. The dual 5α-reductase inhibitor, dutasteride (17β-N-2,5-bis-[trifluoromethyl]-phenylcarbamoyl-4-aza-5a-androstan-1-en-3-one), was from AK Scientific, Inc. (Mountain View, CA) and was >98% pure by HPLC.

2.2. Animals and androgenic assays

Immature male Sprague–Dawley CD rats (Crl:CD(SD)) were purchased from Charles River Laboratories (Kingston, NY) and castrated at 22 days of age. To determine the potency of the 5α-reduced derivatives of DMA and MNT compared to the parent compounds and to T, which is the standard in the sc androgenic assay, rats (8/group) were injected sc daily, starting on the day of castration (day 22) and continuing for 7 additional days, with either vehicle (10% EtOH/sesame oil) or various doses of T, DHT, DMA, 5α-DHDMA, MNT, or 5α-DHMNT. Twenty-four hours after the final dose, the animals were euthanized, and body weights were obtained. The VP, SV, and LA muscle were excised, trimmed, blotted, and weighed to the nearest 0.1 mg [8].

To determine the effect of DUT in the androgenic assay, rats ($n = 10$/group) were initially treated sc with vehicle, T (0.23 mg/day), or T and 0.1 mg/day, 1.0 mg/day, or 10.0 mg/day of DUT orally for 8 days. Thereafter, rats (10/group) were injected sc with either vehicle or maximal stimulatory doses of T, DHT, DMA, MNT, or MENT for 8 consecutive days. Concurrent with the sc injections, vehicle or DUT was administered orally at 1.0 mg/day which was determined to be the optimal dose (see Section 3.3). Two hours after the final dose, the rats were euthanized, blood was collected for measurement of serum androgen levels, and body weights were obtained. The VP and SV were excised, trimmed, blotted, and weighed.

2.3. Radioimmunoassays (RIAs)

Serum levels of DMA and MNT and their putative immunoreactive metabolites were determined using specific RIAs developed at Bioqual, Inc. [5,9]. The limits of detection were 125 pg/ml (5 μl serum) for DMA and 2.37 ng/ml (0.5 μl serum) for MNT. MENT was measured by RIA [10] at the Population Council, courtesy of Dr. N. Kumar. The limit of detection was 130 pg/ml. T and DHT were also measured by RIA using kits from DPC (Coat-a-Count, Los Angeles, CA) and DSL (DSL-9600 ACTIVE® DHT Coated-Tube RIA, Webster, TX), respectively. The limit of detection in the T assay was 60 pg/ml or 70 pg/ml (EC_{90}) and in the DHT assay, 25 pg/ml (lowest standard).

2.4. Androgen receptor binding and transactivation

Binding to androgen receptors (AR) was performed as described previously [11]. Briefly, purified rat AR ligand binding domain (ARLBD; ~200 fmol/tube), purchased from Invitrogen (Carlsbad, CA), was incubated in microfuge tubes with 5 nM 17α-methyl-[^{3}H]R1881 (PerkinElmer, Boston, MA) in 50 mM Tris, pH 7.5, 0.8 M NaCl, 10% glycerol, 0.1% BSA, 0.002% NP-40 in the absence or presence of 5 μM R1881 to measure total and nonspecific binding, respectively. Unlabeled competitors were added at final concentrations from 0.1 to 500 nM. After an overnight incubation at 2-6 °C, [^{3}H]R1881–ARLBD complexes were separated from unbound radioligand by addition of 100 μl 50% hydroxylapatite and centrifugation. Precipitates were washed three times, and bound radioactivity was extracted with 200 μl 95% ethanol and counted in a LKB Wallac Rackbeta scintillation counter. R1881 was the standard. DUT did not bind to the ARLBD within the limits of the concentrations tested ($IC_{50} > 500$ nM).

CV-1 cells (ATCC, Manassas, VA) were used to assess transcriptional activity of the various androgens. Cells were cotransfected with 3XHRE-LUC (containing three copies of a hormone response element fused to the firefly luciferase gene) and a human AR expression plasmid (gifts of Dr. D. Robins, University of Michigan, Ann Arbor, MI), treated for 20 h with vehicle (100% ethanol) or various concentrations of androgens (range 10^{-12} to 10^{-5} M), and harvested. Luciferase activity was measured and normalized as specified previously [5].

2.5. Statistical analysis

Data are expressed as the mean ± SE. Statistical analyses were performed using SigmaStat for Windows (Version 3.50, SPSS Inc., Chicago, IL) or Microsoft Excel (Microsoft Corp.). EC_{50}s in transactivation assays or IC_{50}s in competitive binding assays for the ARLBD were determined using GraphPad PRISM™, version 4.0 (GraphPad Software, San Diego, CA,) and compared statistically by the nonparametric Kruskal–Wallis one way ANOVA on ranks, as the data did not pass normality, followed by Dunn's all pairwise multiple comparison test. Effects of various doses of DMA, 5α-DHDMA, MNT, 5α-DHMNT, and T on VP, SV, and LA muscle weights in the