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Different Patterns of Metabolism Determine the Relative Anabolic Activity of 19-Norandrogens

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Testosterone, the principal androgen secreted by Leydig cells, exerts a wide range of actions including growth of the male reproductive tract (androgenic effects) and growth of non-reproductive tissues such as muscle, kidney, liver, and salivary gland (anabolic effects). As androgenic steroids were discovered some were found to have relatively more anabolic than androgenic activity. The results reviewed in this report suggest that these differences result, in part, from the differential metabolism of the steroids in individual tissues and the varied activities of the individual metabolites. In the accessory sex organs (e.g. the prostate) testosterone is 5α-reduced to dihydrotestosterone (DHT) which, due to its higher affinity for androgen receptors (AR), amplifies the action of testosterone. In contrast, when 19-nortestosterone (NT) is 5α-reduced, its affinity for AR decreases, resulting in a decrease in its androgenic potency. However, their anabolic potency remains unchanged since significant 5α-reduction of the steroids does not occur in the muscle. 7α-methyl-19-nortestosterone (MENT) does not get 5α-reduced due to steric hindrance from the 7α-methyl group. Therefore, the androgenic potency of MENT is not amplified as happens with testosterone. These metabolic differences are responsible for the increased anabolic activity of NT and MENT compared to testosterone. Part of the biological effects of testosterone are mediated by its aromatization to estrogens. The fact that MENT is also aromatized to 7α-methyl estradiol, a potent estrogen, *in vitro* by human placental and rat ovarian aromatase suggests that some of the anabolic actions of MENT may be mediated by this estrogen.

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INTRODUCTION

An androgen is an agent that stimulates growth of the male reproductive tract and external genitalia [1]. In addition, androgens have diverse effects on many organs including muscle, kidney, salivary gland, and liver that have little direct bearing on male characteristics. These latter effects are designated anabolic effects. As various androgenic steroids were discovered it was noted that some were relatively more androgenic and others more anabolic. Such observations fostered programs designed to synthesize anabolic steroids without androgenic activity. The failure to separate androgenic and anabolic activities relates, in part, to the fact that most androgenic and anabolic effects are mediated via the androgen receptor [2]. Thus, the relative potency of androgenic steroids when measured by single end point can be related in part to the affinity of these steroids to androgen receptors [3]. However, it seemed unlikely that the differential effects of such steroids on prostate, muscle, kidney, and liver could be explained by receptor binding alone [4].

Testosterone (T), the principal androgen secreted by the Leydig cells, affects its target organs by interacting with specific androgen receptors. While in many tissues T is the active androgen *per se*, in others, part of the important biological actions of T depend upon its enzymatic conversion to active metabolites [5]. In the male accessory sex organs and skin, the precursor, T, undergoes 5α-reduction to the product, 5α-dihydrotestosterone (DHT) which leads to an amplification of the apparent potency of the precursor [4, 6]. In many tissues, including some parts of the brain, liver and adipose tissue, aromatase [7–9] converts T to estradiol (E_2) that in turn exerts its effects via estrogen receptors.

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Hence, the androgenic and anabolic actions of T are indeed complex. In each tissue the magnitude and quality of the response depends upon the enzymatic activity in that tissue, the active intracellular hormones that are generated, and the receptors that are present. Regardless of the hormone that produces the final response it is, none-the-less, designated androgenic or anabolic depending on the tissue.

Since it is likely that the action of androgens that have been dubbed "anabolic" are likely to be as complex as that of testosterone, it was of interest to determine whether the metabolism of these steroids determines the differential tissue responses of muscle and prostate [10, 11]. It is the purpose of this manuscript to relate the differential metabolism of 19-nor androgens to their biological activities on several organs.

RELATIVE BIOPOTENCY OF ANABOLIC STEROIDS

The androgenic and anabolic potencies of some synthetic 19-nor androgens were compared with that of T in castrated rats. For the purpose of this study, muscle weight was used as an index of anabolic activity while prostate and seminal vesicles weights served as an index of androgenic activity. Multiple doses of each androgen were administered via Alzet osmotic pumps for 14 days and the weights of ventral prostate, seminal vesicles and levator ani were determined. Using an Allfit computer program, the relative potencies of each androgen were estimated (Table 1).

7α-methyl-19-nortestosterone (MENT) was the most potent androgen in this series. Based on the weights of prostate and seminal vesicles, MENT was 4–5 times more potent (androgenic) than T. By contrast; the myotropic potency, estimated from levator ani response, was nearly 10 times that of T. In contrast to MENT, 19-nortestosterone (NT) was 5 times less androgenic than T but twice as anabolic as T. In a similar study, 17α-methyl-19-nortestosterone (17MNT) was found to be less androgenic and more anabolic than 17α-methyl-testosterone (17MT) (Table 1). These observations indicate that NT, 17MNT, and MENT (the nor-androgens) are more anabolic than androgenic, that is, their relative anabolic activities are greater than one (Table 1).

RECEPTOR BINDING AND THE ROLE OF 5α-REDUCTASE

Competitive binding studies to rat prostate androgen receptors showed MENT to be most potent in displacing [^{3}H]mibolorone followed by DHT, NT and T. These results showed that the binding affinities of these steroids were correlated more closely with the effects on muscle. Since the androgen receptor in the muscle is the same as that in the prostate [12–14] we sought to determine if the difference between the androgenic and anabolic actions of these androgens was related to their metabolism in prostate.

As noted above, in the prostate and seminal vesicles T is enzymatically 5α-reduced to DHT whose binding affinity to AR and bioactivity on the tissues is 3–5 times greater than that of T. These events lead to a 2–3-fold amplification of T action in these tissues. On the other hand, muscle contains very little or no functional 5α-reductase enzyme [4, 15, 16]. Hence the response of the muscle reflects the inherent activity of the unmodified testosterone or another metabolite. NT, which has higher affinity for androgen receptors than T, also exhibits a greater myotropic potency. However, dihydro-NT, the 5α-reduced metabolite of NT, has lower affinity for AR than NT, T and DHT [17, 18]. These findings suggest that in contrast to T, whose action is amplified in prostate by its conversion to DHT, the potency of NT on the prostate is reduced by its 5α-reduction to dihydro-NT [19]. If this explanation is correct then a 5α-reductase inhibitor should have strikingly different effects on the actions of T and NT. This possibility was investigated.

Castrated rats were treated with T or NT with or

Table 1. *Relative biopotency estimates of androgens in castrated rats*

Steroids	Potency estimates based on weights of			
	Ventral prostate	Seminal vesicles	Levator ani	Relative anabolic activity*
Experiment A				
Testosterone (T)	1	1	1	1
7α-methyl-19-nortestosterone (MENT)	4	5	9	2
19-nortestosterone (NT)	0.2	0.2	2.4	12
Experiment B				
17α-methyl-testosterone	1	1	1	1
17α-methyl-19-nortestosterone	0.2	0.3	2.3	11

Androgen treatment was started on the day of castration. The duration of treatment was 14 days. Potency estimated by "Allfit" computer program. The comparisons of testosterone, MENT and nortestosterone were performed in one study and the 17-methylated androgens in another. In Experiment A, the potency of testosterone was assigned a value of one. In Experiment B, 17α-methyl-testosterone was assigned a value of one.

*Relative anabolic activity = potency on muscle divided by potency on prostate.

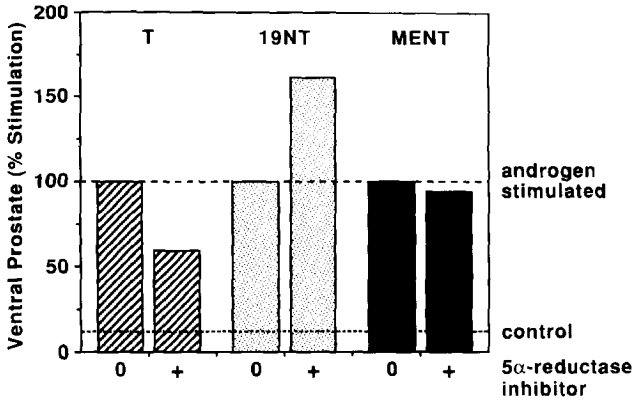

Fig. 1. Effect of 5α-reductase inhibitor on the androgenic activity of T, NT and MENT in castrated rats. The response to the androgens is designated 100.

without the 5α-reductase inhibitor, *N*,*N*-diethyl-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide. In castrated rats receiving T, this compound inhibited the stimulatory action of T on the prostate (Fig. 1) as expected but did not affect its action on muscle (not shown). On the other hand, the 5α-reductase inhibitor increased the prostate weights in rats receiving NT (Fig. 1) without affecting the effect on muscle (not shown). In a similar study the 5α-reductase inhibitor decreased the androgenic potency of 17MT and increased the androgenic potency of 17MNT without altering their anabolic potencies [11]. These findings are consistent with the postulate that the androgenic action of these steroids is dependent upon the relative biopotency of their 5α-reduced product generated in the prostate. The latter studies also indicate that the 17α-methyl group does not modify the A-ring metabolism of 17MNT by 5α-reductase. As a consequence, the relative anabolic activities of NT and 17MNT are similar (Table 1) due to the reduced androgenic activity that results as a consequence of their 5α-reduction.

MENT IS RESISTANT TO 5α-REDUCTION

The dissociation of the androgenic and anabolic activity of MENT is also related to its metabolic transformation in the prostate. The *in vitro* metabolism of MENT by rat liver and prostate was investigated [20]. Radioactively labelled MENT incubated with liver homogenates yielded three metabolites: 7α-methyl-estr-4-ene-3,7-dione; 7α-methyl-5β-estrane-3,17β-diol; and 7α-methyl-3-oxo-estr-4-ene-16,17β-diol (Fig. 2). There was no evidence of 5α-reduced products. In another study, there was no detectable metabolism of MENT by prostate homogenates. In parallel investigations, 48% of radiolabelled T was converted to 5α-reduced products by the prostate. We concluded from these observations that MENT did not undergo 5α-reduction, probably because the 7α-methyl group hinders the action of 5α-reductase.

Studies on the metabolism of MENT led to the prediction that, compared to T, the relative biopotency of MENT on the prostate would be lower than that on muscle. The relative biopotency estimates of MENT shown in Table 1 are consistent with this hypothesis. Further support for this conclusion was provided in experiments with 5α-reductase inhibitor [21, 22]. Castrated rats were treated with T and MENT with or without the inhibitor. As expected, the enzyme inhibitor attenuated the action of T on the prostate (Fig. 1) but not on muscle (not shown). As predicted, the inhibitor did not alter the action of MENT on prostate (Fig. 1) or muscle. This shows that the partial dissociation of the androgenic and anabolic actions of MENT relates to its lack of amplification in the prostate by 5α-reductase. Studies of the metabolism of nor-androgens from our own and other laboratories suggested that the 7α-methyl group was responsible for rendering MENT resistant to 5α-reduction. To investigate this possibility, other androgens with 7α-substitution were studied. Like MENT, these steroids were

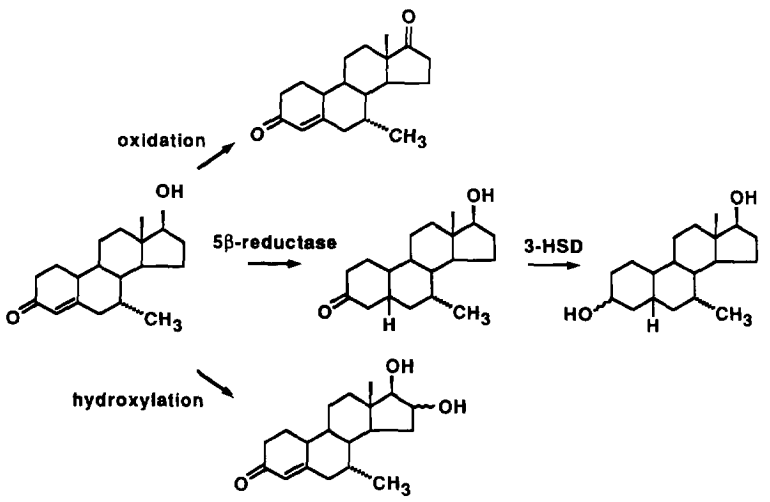

Fig. 2. Proposed pathway of MENT metabolism by male rat liver (from Ref. [20]).

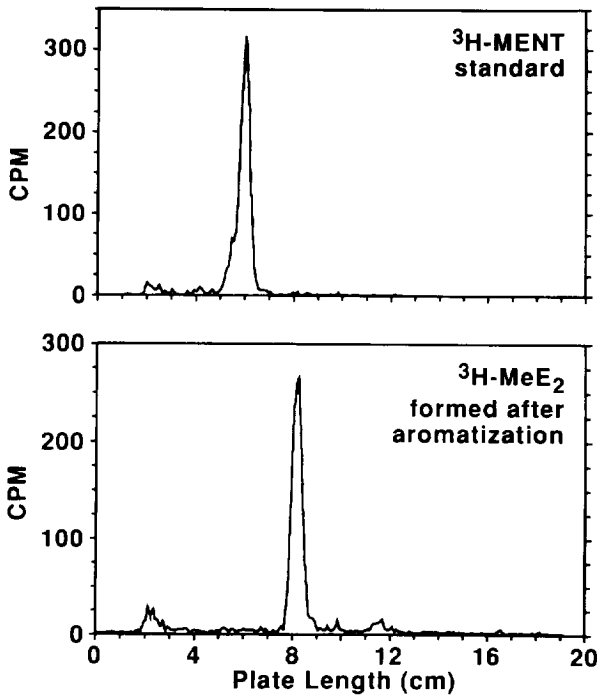

Fig. 3. Radiochromatogram of the 0.4 N NaOH extract following incubation of [^{3}H]MENT with rat ovarian microsomes. Following incubation, the steroids were extracted and partitioned between benzene/petroleum ether and 0.4 N NaOH. Methylene chloride:ethylacetate (8:2) was used as the mobile phase. The scan shows [^{3}H]MENT standard (top) and its aromatized derivative (bottom). The mobility of the aromatized derivative was similar to that of non-radioactive 7α-methyl-estradiol standard visualized under UV light.

also more anabolic relative to testosterone [21]. These observations led to the proposal that MENT could be used for androgen replacement therapy in men at a dose that would maintain muscle mass but would not stimulate prostate growth [22].

AROMATIZATION

Since T undergoes enzymatic aromatization to E_2 in many tissues, some of the actions of T in the male have been attributed to its metabolite, E_2, including the regulation of gonadotropin secretion and sexual behavior [23–25] and possibly indirect anabolic response through stimulation of growth hormone and/or insulin-like growth factor-I [26]. Therefore, studies were undertaken to determine whether MENT could be aromatized. Human placental microsomes were used as the source of aromatase [27].

[^{3}H]MENT and microsomes were incubated at 37°C in the presence of NADPH for 60 min. The metabolites were partitioned between benzene-petroleum ether and 0.4 N NaOH, and the radioactivity in the two phases was measured (Table 2). The results show that placental microsomes convert MENT into a product that can be partitioned into NaOH and that this conversion is blocked by an aromatase inhibitor. The conversion of MENT to estrogen was further confirmed by thin-layer chromatography of the NaOH fraction. The dominant metabolite had the mobility of 7α-methyl-estradiol (7α-methyl-E_2), a product with estrogenic activity [27]. Moslemi *et al.* [28] reported that MENT was aromatized by equine placental microsomes but not by human placental microsomes. The reason for the discrepancy between the two studies is not clear at the present. Using similar techniques we have also observed that MENT was aromatized by rat ovarian microsomes *in vitro*.

Since MENT was shown to be aromatized to estrogenic compounds, the estrogenic activity of MENT was investigated. Competitive binding studies with rat uterine estrogen receptors showed that MENT had low but significant affinity for estrogen receptors while testosterone showed very little binding [27]. The ED_{50} of MENT was approx. 3000-fold higher than that of E_2. *In vivo* studies in ovariectomized female rats showed that MENT caused a dose-dependent increase in uterine weight with MENT being over 50-fold more potent than testosterone but with 0.005% activity of E_2 in the uterotropic assay. Examination of vaginal histology showed no evidence of cornification similar to that seen with estradiol. It would seem that the anabolic action of MENT accounts for most of its uterotropic activity and is mediated through binding to AR. Support for this theory was provided by studies in which the uterotropic activity of MENT was partially inhibited by cyproterone, an antiandrogen.

These studies show that there are several mechanisms involved in the anabolic actions of 19-norandrogens. These include: (1) differences in the binding affinity to androgen receptors; (2) differences in suscep-

Table 2. Metabolism of [^{3}H]MENT by human placental microsomes (Mic)

	cpm in base ($\times 10^{-3}$)	cpm in BP ($\times 10^{-3}$)
[^{3}H]MENT	44	1027
[^{3}H]MENT + Mic + NADPH	827	89
[^{3}H]MENT + Mic − NADPH	36	728
[^{3}H]MENT + Mic + NADPH + R76713	55	711

Tritiated MENT (in duplicate) was incubated with placental microsomes with or without additives for 60 min. The products were extracted and partitioned between 0.4 N NaOH (base) and benzene:petroleum ether (BP) and the total radioactivity in each fraction determined (from Ref. [27]).

tibility to 5α-reduction; and (3) differences in the biopotency of 5α-reduced metabolites.

In conclusion, we have shown that the differential susceptibility of 19 nor-androgens to 5α-reduction determines their affinities to the androgen receptors leading to a dissociation of androgenic and anabolic activities. These findings provide a rational basis for understanding how MENT has increased anabolic activity compared to testosterone.

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