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Pretranslational Up-regulation of the Hepatic Microsomal Δ^4-3-Oxosteroid 5α-Oxidoreductase in Male Rat Liver by all-*trans*-Retinoic Acid

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ABSTRACT. Administration of all-*trans*-retinoic acid (ATRA; 60 mg/kg daily for 3 days) to male rats increased the rate of 5α-dihydrotestosterone (5α-DHT) formation from testosterone in microsomal fractions *in vitro*. The formation of androstane-3α,17β-diol from testosterone was also increased because of the higher concentration of 5α-DHT produced in microsomal incubations. Northern analysis confirmed that the increased rate of 5α-DHT formation was due to the pretranslational up-regulation in Δ^4-3-oxosteroid 5α-oxidoreductase (EC 1.3.99.5) mRNA expression in ATRA-treated male rat liver. Thus, ATRA elicited in male rat liver a partial feminization of the expression of this enzyme, which normally exhibits a female-selective distribution in the rat. Subsequent experiments evaluated whether the administration of human chorionic gonadotropin or thyroxine to ATRA-treated male rats decreases 5α-reductase activity to that observed in untreated male rat liver. Although these treatments did not decrease 5α-reductase to untreated male levels, it was found that administration of ATRA to gonadectomized male rats produced complete feminization of the enzyme. Again, up-regulation was confirmed at the mRNA level. The activity of the male-specific cytochrome P450 2C11 (as reflected by microsomal testosterone 16α-hydroxylation activity) was correspondingly decreased by treatments that increased steroid 5α-reductase activity. Thus, gonadectomy in combination with ATRA administration effected a more pronounced decrease in 16α-hydroxylation activity than either treatment alone. These findings suggest that ATRA is a novel positive regulator of the 5α-reductase that in combination with the removal of circulating androgen, which normally suppresses 5α-reductase levels, feminizes the expression of this enzyme in rat liver. BIOCHEM PHARMACOL **58**;2:355–362, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. all-*trans*-retinoic acid; Δ^4-3-oxosteroid 5α-oxidoreductase; hepatic enzyme regulation; steroid biotransformation; vitamin A; dihydrotestosterone

The hepatic microsomal 5α-reductase† (EC 1.3.99.5) catalyzes the reduction of the 4,5-olefinic bond in androgens such as testosterone (Fig. 1). Two distinct genes code for the individual isoforms of the 5α-reductase, which are expressed in a tissue-specific manner [1]. Thus, 5α-reductase type 1 is highly expressed in liver, especially in the female rat, and the type 2 enzyme is distributed throughout the male reproductive system in androgen-responsive tissues. The type 2 enzyme is thought to have an important role in androgen activation, but the role of the type 1 enzyme is less clear, although it may influence androgen elimination [2]. Studies in mice deficient in the type 1 5α-reductase following gene disruption have suggested that the enzyme may protect the animal against estrogen toxicity [3].

ATRA and other retinoids are important regulators of gene transcription mediated via retinoid receptors [4]. In the clinical setting, ATRA is valuable in the treatment of several types of malignant disease, including acute promyelocytic leukemia [5]. Although the mechanism of these beneficial therapeutic effects remains unclear, it may involve the role of ATRA in differentiation. However, treatment with ATRA ultimately fails and precipitates retinoid resistance, which is characterized by enhanced rates of ATRA elimination, and is consistent with the up-regulation of hepatic drug-metabolizing enzymes [6, 7].

Vitamin A status also influences the expression and function of drug-metabolizing enzymes in mammalian liver. In previous studies, we have demonstrated the down-regulation of the androgen-dependent male-specific CYP2C11 in rat liver by intake of a vitamin A-deficient diet [8]. Although inclusion of ATRA at low levels in diet restores the enzyme, exogenous ATRA administration by i.p. injection decreases CYP2C11 expression in male rat liver [9]. Thus, retinoids participate in the maintenance of CYP2C11 but do not directly activate the transcription of this gene in rat liver.

Because the 5α-reductase and CYP2C11 appear subject

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† *Abbreviations:* ATRA, all-*trans*-retinoic acid; CYP, cytochrome P450; 5α-DHT, 5α-dihydrotestosterone; hCG, human chorionic gonadotropin; MT, methyltrienolone; T_4, thyroxine; 5α-reductase, Δ^4-3-oxosteroid 5α-oxidoreductase; 3α,17β-diol, 5α-androstane-3α,17β-diol; and 3β,17β-diol, 5α-androstane-3β,17β-diol.

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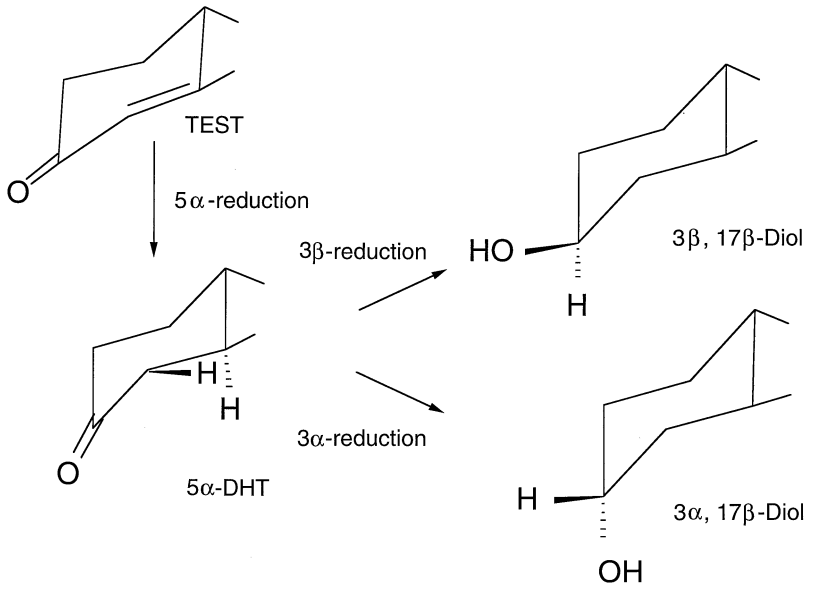

FIG. 1. Pathways of steroid A-ring reduction in rat hepatic microsomes. The example of A-ring biotransformation of testosterone (TEST) is presented showing initial 5α-reduction (to 5α-DHT) and subsequent 3α/3β-reduction to form the epimeric alcohols 3α,17β-diol and 3β,17β-diol.

to reciprocal regulation, the present study evaluated whether the down-regulation of CYP2C11 that has been observed in ATRA-treated male rats may occur concurrently with up-regulation of the 5α-reductase. The principal finding to emerge from this study was that administration of ATRA increased hepatic 5α-reductase activity and the expression of its corresponding mRNA. Complete feminization of 5α-reductase function and expression was achieved by administration of ATRA to androgen-depleted (gonadectomized) animals. Thus, ATRA appears to be a novel positive regulator of the microsomal 5α-reductase, although the repressive effects of androgen or androgen-dependent factors take precedence in intact male rats.

MATERIALS AND METHODS

Chemicals

[^{14}C]Testosterone (sp. act. 59 mCi/mmol), [^{14}C]5α-DHT (sp. act. 56 mCi/mmol), [α-^{32}P]dCTP (sp. act. 3000 Ci/mmol), Hyperfilm-MP, ACS II, Hybond-N$^+$ filters, and reagents for enhanced chemiluminescence were purchased from Amersham Australia. ATRA, hCG, T_4, steroid standards, and biochemicals were from the Sigma Chemical Co. MT was purchased from New England Nuclear. Reagents for sodium dodecyl sulfate-polyacrylamide gel electrophoresis were obtained from Bio-Rad Laboratories. HPLC grade solvents were obtained from Rhone-Poulenc Chemicals. Analytical reagent grade solvents and other chemicals were purchased from Ajax.

Animal Treatments

Experiments were approved by the Institutional Animal Care and Ethics Committee. Male Wistar rats (~250 g) were obtained from the in-house breeding colony and were held in wire cages at constant temperature and lighting (12-hr light–dark cycle). In initial experiments, rats were administered ATRA by i.p. injection (60 mg/kg in corn oil daily for 3 days and were killed 24 hr after the third dose). In subsequent experiments, animals received ATRA; some also received either MT (0.625 mg/kg in propylene glycol by s.c. injection once daily for 5 days) [10], hCG (500 U/kg in 10 mM potassium phosphate buffer, pH 7.2, by s.c. injection once daily for 7 days) [10], or T_4 (50 mg/kg in 10 mM potassium phosphate buffered-saline, pH 8.3, by s.c. injection once daily for 7 days) [11].

At the end of the experimental periods, animals were anesthetized and killed. Livers were removed and perfused with ice-cold saline, and part of the tissue was frozen in liquid nitrogen for determination of hepatic 5α-reductase type 1 mRNA. Washed hepatic microsomes were prepared from the remainder of the tissue by differential ultracentrifugation, resuspended in 50 mM potassium phosphate buffer, pH 7.4, containing 20% glycerol and 1 mM EDTA, snap frozen in liquid nitrogen, and stored at −70° until required in experiments [12]. Microsomal protein was estimated by the procedure of Lowry *et al.* [13].

Microsomal Steroid Biotransformation

In preliminary studies it was noted that the activity of the hepatic microsomal 5α-reductase was inactivated rapidly at 37° in the presence of steroid substrate ($T_{1/2}$ ~4 min). This was overcome by combining reagents that had been held at 37° and initiating the reaction immediately. Incubation of testosterone or 5α-DHT (50 μM; 0.18 μCi/0.4 mL reaction volume) with 0.15 mg microsomal protein in potassium

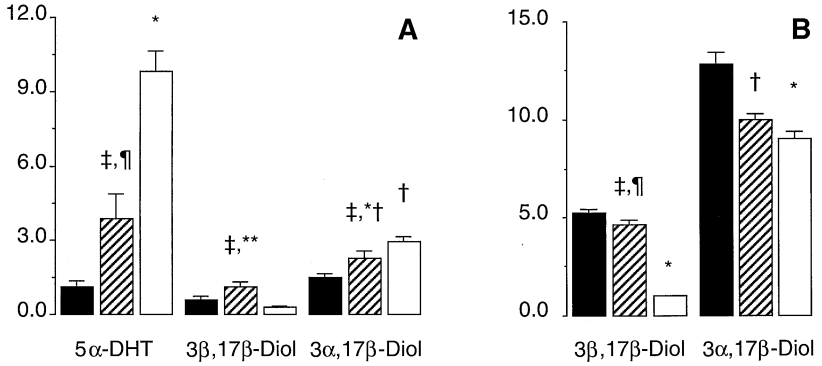

FIG. 2. Formation of A-ring reduced metabolites in hepatic microsomes from untreated male (closed bars), ATRA-treated male (diagonal hatching), and untreated female (open bars) rats; (A) testosterone as substrate, and (B) 5α-DHT as substrate. Units: nmol/min/mg protein. Data are means ± SEM, N = 3. Key: significantly different from untreated male rat: (*) $P < 0.001$, (†) $P < 0.01$, and (‡) $P < 0.05$; significantly different from untreated female rats: (¶) $P < 0.001$, (**) $P < 0.01$, and (*†) $P < 0.05$.

phosphate buffer (0.1 M, pH 7.4, containing 1 mM EDTA) was conducted for 2.5 min at 37° [14]. The reactions were initiated by the addition of NADH or NADPH (1 mM final concentration) and were terminated by the addition of 5 mL of chloroform and removal to ice. After extraction, centrifugation, and separation, the organic phase was evaporated to dryness under N_2, and the residue was applied to TLC plates (Merck silica gel 60 F_{254} type). The plates were run six times in chloroform:ethyl acetate, 6:1 (NADH reactions, which enabled the resolution of 3α,17β- and 3β,17β-diols), or sequentially in dichloromethane:acetone, 4:1, and then chloroform:ethyl acetate:ethanol, 4:1:0.7 (NADPH reactions [15]), with air drying between. Radioactive metabolites were located by autoradiography (Hyperfilm-MP; Amersham) over ~60 hr or by heating the TLC plates to 120° for 5 min after treatment with 10% phosphomolybdic acid in ethanol. Metabolite formation was quantified by scintillation counting in ACS II (Amersham).

RNA Analysis

Total RNA was extracted from male rat liver by the method of Sambrook *et al.* [16]. For analysis of the hepatic 5α-reductase mRNA, the synthetic 22-mer oligonucleotide described previously [14], and complementary to nucleotides 793–814 of the published cDNA sequence [17], was employed. The oligonucleotide was labeled using [α-^{32}P]dCTP and deoxynucleotidyl transferase. To demonstrate equivalence of RNA loading between samples, filters were stripped and rehybridized to the [α-^{32}P]-labeled 18S ribosomal RNA probe [18].

In northern analysis, total RNA (20 μg) was electrophoresed on 1.2% agarose in the presence of 2.2 M formaldehyde and then transferred to Hybond-N^+ nylon filters (0.45 μm, Amersham) [16]. Hybridization, washing, and autoradiographic conditions on Hyperfilm-MP were as described previously [14]. After autoradiography, the filters were stripped and rehybridized to the 18S ribosomal RNA [α-^{32}P]-labeled probe. In the case of slot blotting, equivalence of RNA loading between samples was confirmed similarly with [α-^{32}P]-labeled β-actin cDNA. After densitometry, signals were quantified using a Molecular Dynamics PhosphorImager.

Statistics

Differences between means from two treatment groups were detected using Student's *t*-test. Differences between means from more than two treatment groups were detected using one-way analysis of variance in conjunction with Dunnett's test (for comparisons to a single control mean) or the Student–Newman–Keuls test (for comparisons between multiple group means) as appropriate.

RESULTS

Effect of In Vivo *ATRA Administration on the Microsomal Reduction of Testosterone to 5α-DHT in Rat Liver*

The administration of ATRA (60 mg/kg, i.p., daily for 3 days) to male rats increased the rate of NADPH- and NADH-mediated microsomal 5α-DHT formation from testosterone to around 3-fold of control ($P < 0.05$; data not shown). NADH-supported formation of 3α,17β-diol was also increased by ATRA treatment ($P < 0.05$), whereas 3β,17β-diol formation was slightly, but not significantly, increased (not shown).

The microsomal 5α-reduction of steroids like testosterone is more extensive in female than in male rat liver; this gender difference was approximately 8.8-fold in the present study (Fig. 2A). Consistent with earlier findings, ATRA administration to male rats increased the activity of the pathway about 3.5-fold (3.87 ± 0.99 vs 1.12 ± 0.23 nmol 5α-DHT formed/min/mg protein; $P < 0.05$), but not to the levels seen in female rat liver microsomes (9.85 ± 0.82 nmol 5α-DHT formed/min/mg protein). Because 5α-DHT formation precedes that of the epimeric 3-alcohols (3α, 17β-diol and 3β,17β-diol), measurements with testosterone alone do not provide information on whether the

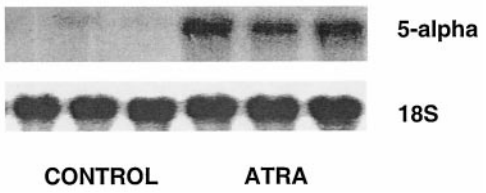

FIG. 3. Northern analysis for 5α-reductase mRNA in liver from three individual untreated control male rats (control) and three individual ATRA-treated male rats (ATRA). Rats were killed 24 hr after the final dose of ATRA. Equivalent loading of mRNA was confirmed by hybridization of the filters to an 18S RNA probe.

3α- or 3β-hydroxysteroid oxidoreductases are also modulated by ATRA. Accordingly, the activities of these enzymes were determined directly, with [^{14}C]5α-DHT as the substrate. As shown in Fig. 2B, both activities, especially that of the 3β-hydroxysteroid oxidoreductase, were greater in male than in female rat liver, but were decreased slightly by ATRA treatment. Thus, the increased rates of conversion of 5α-DHT to the 3α,17β- and 3β,17β-diols that were noted after ATRA administration occurred indirectly as a consequence of enhanced 5α-reductase activity.

5α-Reductase activity was measured in hepatic microsomes from rats that were killed 1, 3, and 5 days after the ATRA treatment regimen. The activity remained elevated 5 days after the final dose of ATRA (not shown). From northern analysis it was found that hepatic expression of the 5α-reductase was up-regulated by ATRA at a pretranslational level (to 7.4-fold of control 24 hr after the final dose of ATRA; $P < 0.01$; Fig. 3).

Effect of Hormonal Manipulation on the Induction of 5α-Reductase Activity by ATRA

Interplay among hormonal mediators has been shown to regulate the microsomal 5α-reductase in rat liver [2, 11]. From Table 1 it is apparent that the increases in 5α-reductase activity produced by ATRA treatment and gonadectomy were similar (to 3.8- and 4.5-fold of control, respectively). Androgen administration to gonadectomized male rats partially reversed the effect of gonadectomy on

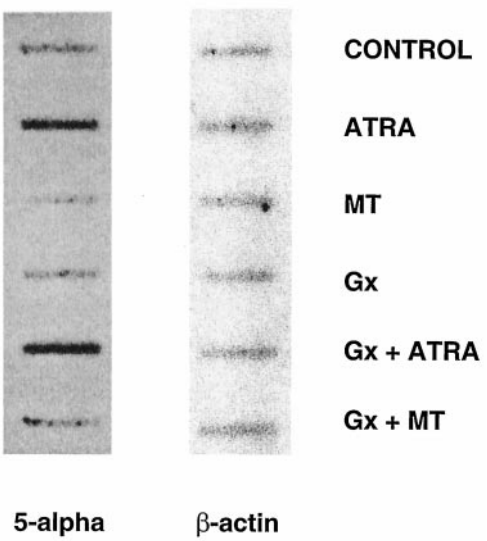

FIG. 4. Slot blot analysis for 5α-reductase mRNA in liver from variously treated male rats: intact control, ATRA-treated intact, MT-treated intact, gonadectomized (Gx), gonadectomized that received ATRA (Gx + ATRA), and gonadectomized that received MT (Gx + MT). Rats were killed 24 hr after the final dose of ATRA. Equivalent loading of mRNA was confirmed by hybridization of the filters to β-actin cDNA probe.

5α-reductase activity (Table 1). However, a major finding was that the administration of ATRA (three doses on consecutive days) to gonadectomized male rats increased 5α-reductase activity to levels found in female rat liver (9.37 ± 0.72 compared with 9.85 ± 0.82 nmol 5α-DHT formed/min/mg protein; Table 1, Fig. 2). In accord with this observation, the signal for the 5α-reductase mRNA was increased in liver of gonadectomized male rats that had also received ATRA (Fig. 4).

Comparative Effects of ATRA Treatment on NADPH-Dependent Testosterone Biotransformation in Rat Hepatic Microsomes

NADPH-supported 5α-DHT formation from testosterone followed the trends observed previously when NADH was used as cofactor, but product formation was somewhat less

TABLE 1. NADH-mediated testosterone biotransformation in hepatic microsomes from intact and gonadectomized male rats treated with MT or ATRA

	5α-DHT	3β,17β-Diol	3α,17β-Diol
Treatment		**(nmol/mg protein/min)**	
Control	1.17 ± 0.32	0.54 ± 0.12	1.38 ± 0.20
Control + MT	1.08 ± 0.14	0.56 ± 0.05	1.45 ± 0.14
Control + ATRA	4.46 ± 1.12*	1.23 ± 0.22	2.44 ± 0.30†
Gonadectomy	5.22 ± 0.46*	0.91 ± 0.14	2.73 ± 0.13*
Gonadectomy + MT	3.09 ± 0.40†	0.89 ± 0.14	2.03 ± 0.19
Gonadectomy + ATRA	9.37 ± 0.72*,‡	0.79 ± 0.19	2.57 ± 0.19*,§

The values represent means ± SEM (N = 3 animals in each group).
*,† Significantly different from control: *$P < 0.01$, and $^{\dagger}P < 0.05$.
‡,§ Significantly different from gonadectomy: $^{\ddagger}P < 0.01$, and $^{\S}P < 0.05$.

TABLE 2. NADPH-mediated testosterone biotransformation in hepatic microsomes from intact and gonadectomized male rats treated with MT or ATRA

Treatment	Testosterone metabolite (nmol/mg protein/min)					
	Androstenedione	5α-DHT	2α-Hydroxy	6β-Hydroxy	7α-Hydroxy	16α-Hydroxy
Control	1.39 ± 0.09	0.45 ± 0.14	2.04 ± 0.27	2.10 ± 0.07	0.36 ± 0.04	2.35 ± 0.33
Control + MT	1.30 ± 0.06	0.44 ± 0.04	2.13 ± 0.25	1.95 ± 0.25	0.36 ± 0.05	2.28 ± 0.32
Control + ATRA	0.79 ± 0.08*	2.13 ± 0.51†	0.81 ± 0.07†	1.43 ± 0.20	0.30 ± 0.05	0.95 ± 0.06*
Gonadectomy	0.97 ± 0.01†	1.80 ± 0.15†	0.98 ± 0.11†	0.53 ± 0.19†	0.47 ± 0.04	1.08 ± 0.07†
Gonadectomy + MT	1.13 ± 0.09	1.07 ± 0.16	1.41 ± 0.22‡	1.30 ± 0.36§	0.44 ± 0.06	1.58 ± 0.23‡
Gonadectomy + ATRA	0.65 ± 0.06*,§	3.67 ± 0.19*,‖	0.43 ± 0.12*	0.32 ± 0.06†	0.23 ± 0.03§	0.56 ± 0.12*

Values represent means ± SEM (N = 3 animals in each group).
*–‡ Significantly different from control: *$P < 0.001$, †$P < 0.01$, and ‡$P < 0.05$.
§,‖ Significantly different from gonadectomy: §$P < 0.05$, and ‖ $P < 0.001$.

extensive with NADPH (Table 2). These data also indicate that ATRA administration decreased NADPH-dependent steroid hydroxylation by CYPs in rat liver. Thus, consistent with earlier findings [9], significant decreases in rates of CYP2C11-dependent testosterone 2α-hydroxylation, 16α-hydroxylation, and androstenedione formation were produced by ATRA; the decline in 6β-hydroxylation did not attain statistical significance ($P \sim 0.10$), due to the variation in the activity between individual animals. Similar effects on these pathways were observed after gonadectomy, although the decline in hepatic CYP3A-mediated steroid 6β-hydroxylation was to 25% of intact control (0.53 ± 0.19 vs 2.10 ± 0.07 nmol/min/mg protein; $P < 0.01$). As shown in Table 2, the combined effect of gonadectomy and ATRA administration on CYP-mediated steroid hydroxylation pathways was pronounced (to 21, 15, 64, 24, and 47% of intact control for 2α-, 6β-, 7α-, and 16α-hydroxylation and androstenedione formation, respectively).

Effects of Other Hormones and Chemicals on 5α-Reductase Activity in Male Rat Liver

Pituitary hormones also influence 5α-reductase expression in rat liver [11, 19]. In further studies, we attempted to modulate hepatic 5α-reductase activity hormonally in male rats that had been administered ATRA. Treatment of rats with the combination of ATRA and gonadotropin (hCG) produced increases of microsomal 5α-reductase activity that were similar to ATRA alone (Table 3). Thus, deficiencies in the secretion of pituitary gonadotropins or hypothalamic releasing factors that could give rise to decreased testicular androgen output are not contributors to the ATRA-mediated increase in 5α-reductase activity in male rat liver. A similar experiment with T_4 replacement also was ineffective, although T_4 treatment alone produced an approximate 70% increase in the activity over that measured in intact control male rat liver (Table 3). It is clear, therefore, that the observed effect of ATRA on steroid 5α-reductase activity is not due to an impairment of thyroid function.

The possibility that lipophilic chemicals, such as ATRA, may elicit non-specific effects on pituitary regulation of the hepatic 5α-reductase was considered. However, administration of several lipophilic agents that are established inducers of CYP, including phenobarbital, dexamethasone, and β-naphthoflavone, had no effect on 5α-DHT formation from testosterone. Similarly, toxic chemicals, including the pesticide parathion, were without effect on the activity (data not shown).

TABLE 3. NADH-mediated testosterone biotransformation in hepatic microsomes from male rats treated with ATRA and gonadotropin or thyroid hormone *in vivo*

Treatment	5α-DHT	3β,17β-Diol	3α,17β-Diol
	(nmol/mg protein/min)		
Experiment 1			
Control	0.95 ± 0.19	0.49 ± 0.05	1.13 ± 0.17
ATRA	3.66 ± 0.48*	0.81 ± 0.03	2.23 ± 0.17*
hCG	0.84 ± 0.09	0.70 ± 0.18	1.15 ± 0.10
ATRA + hCG	4.00 ± 1.36*	1.31 ± 0.36	2.40 ± 0.43*
Experiment 2			
Control	1.52 ± 0.37	0.38 ± 0.05	1.49 ± 0.24
ATRA	4.74 ± 0.01†	0.52 ± 0.05	2.33 ± 0.17*
T_4	2.57 ± 0.26*	0.53 ± 0.07	1.71 ± 0.10
ATRA + T_4	5.36 ± 0.02†	0.61 ± 0.15	2.12 ± 0.05*

Values represent means ± SEM (N = 3 animals in each group).
*,† Significantly different from control: *$P < 0.05$, and †$P < 0.01$.

DISCUSSION

The hormonal regulation of the 5α-reductase has been studied intensively by several groups [2, 11, 19, 20]. The enzyme is expressed at a higher level in female than in male rat liver, and gonadal hormones have been implicated in its regulation. Thus, in the male rat, gonadectomy increases the activity of this enzyme in liver, but not to the levels detected in female rat liver, and the increase can be partially reversed by androgen treatment ([20] and this study). Estrogen administration increases steroid 5α-reduction activity in liver of gonadectomized and intact male rats [2]. Thus, androgen is implicated in repression of the 5α-reductase in adult males. In females, ovariectomy influences 5α-reductase expression to a small extent, which suggests that estrogen is not a dominant positive regulator of the enzyme [2, 21]. The present study complements these findings and implicates ATRA in the positive regulation of the 5α-reductase. Thus, partial feminization of the activity, similar to the effect of gonadectomy, was produced by ATRA administration over 3 days. Complete feminization was apparent if the animals had undergone gonadectomy prior to treatment with ATRA.

Part of the regulatory actions of gonadal steroids on hepatic drug-metabolizing enzymes are mediated via the hypothalamic-pituitary-gonadal axis. Hypophysectomy removes the gender difference in 5α-reductase expression so that the activity in female rat liver declines to that in male rat liver [11]. The weight of experimental evidence suggests that the pattern of growth hormone release by the pituitary is a pivotal factor responsible for gender-related differences in the expression of hepatic enzymes in the rat [22, 23]. Gonadal steroids can act at the hypothalamus to influence growth hormone releasing factor, which, in turn, modulates the release of growth hormone by the pituitary. Although growth hormone has an important regulatory role, only partial restoration of the 5α-reductase was produced in hypophysectomized rats that were administered growth hormone [11]. Instead, T_4 acts in concert with growth hormone to regulate the 5α-reductase. This was most evident in rats that were rendered hypothyroid by methimazole treatment and in which the combined effect of T_4 and growth hormone replacement on 5α-reductase activity appeared synergistic [11]. The present studies were undertaken for the most part in intact animals, although some male rats underwent gonadectomy to relieve the androgen-mediated repression of 5α-reductase. ATRA has emerged as another humoral mediator with the capacity to regulate the expression of the 5α-reductase at a pretranslational level.

Numerous studies have described the apparent reciprocal regulation of the 5α-reductase and CYP2C11 in rat liver: the former is present at a much higher concentration in female rat liver, whereas the converse is true for CYP2C11 [2, 19, 24]. During this study, the activity of CYP2C11 was monitored as a biochemical reference marker for gender effects on hepatic steroid metabolism. Essentially it was found that, in keeping with previous studies, CYP2C11 was regulated in reciprocal fashion to the 5α-reductase. Thus, gonadectomy and ATRA treatment, as well as combined gonadectomy and ATRA, increased the rate of NADH-supported 5α-DHT formation from testosterone and decreased the rate of NADPH-supported steroid 16α-hydroxylation mediated by CYP2C11. Indeed, this is reflected by the ratio of the two activities (5α-DHT:16α-hydroxytestosterone), which was 0.50 in microsomal fractions from control rats and was increased to 4.7 and 4.8 in microsomes from ATRA-treated and gonadectomized male rats, respectively. The combined effect of ATRA and gonadectomy was to increase this ratio to 17. By comparison, the ratio was found to be 48 in microsomes from untreated female rat liver, as a result of the low level of steroid 16α-hydroxylation in these fractions (0.20 nmol/min/mg protein; not shown).

The 5α-reductase is active on C19- and C21-steroids, but not steroids with aromatic or saturated A-rings. In the male rat, 5α-DHT, the product of the action of the type 1 5α-reductase on testosterone [1], is perhaps the most biologically important androgen *in vivo*. However, it is unlikely that the hepatic enzyme contributes significantly to androgen action because the 5α-DHT is produced in close proximity to highly efficient enzymes that are active in the hydroxylation [20] and conjugation [25] of A-ring reduced steroids. It is conceivable that A-ring reduction in liver may preclude the aromatization of androgenic precursors and serve to restrict estrogen formation.

NADPH has been commonly employed as the cofactor for the 5α-reductase since the early reports that NADH did not support dihydrotestosterone formation from testosterone in liver homogenates [26]. However, several reports have documented high NADH-mediated activity in microsomes from different mammalian species [27–29]. An explanation to account for this discrepancy arose from the work of Leybold and Staudinger [27], who demonstrated that phosphate is essential for measurable NADH-dependent microsomal dihydrotestosterone formation. It has been suggested that different enzymes may mediate the NADPH- and NADH-linked activities, but the evidence for this is equivocal because it stems largely from differential effects of chemical inhibitors and pH on the activities [28, 29]. Indeed, the two human 5α-reductase genes that have been described to date, and that are expressed in liver, both employ NADPH [30]. Instead, in the present study, the effects of gonadectomy, and androgen or ATRA treatment of rats were similar with either cofactor, and ATRA was found to up-regulate the 5α-reductase mRNA.

Evidence that retinoids and androgens may be substrates for common enzymes has emerged recently. Retinol dehydrogenation to retinal, a prerequisite for the intracellular formation of ATRA, and dehydrogenation of 3α-hydroxysteroids can be catalyzed by several members of the *cis*-retinol/androgen dehydrogenase (CRAD) subfamily [31–33]. Thus, interactions between retinoids and androgens may occur at the level of substrate biotransformation as well as at the level of gene regulation.

ATRA is in increasing use in the management of several forms of cancer, even though prolonged therapy usually fails and leads to retinoid resistance [6, 7]. Like ATRA, administration of certain other cytotoxic agents, including cisplatin [10] and cyclophosphamide [34], has been shown to increase the 5α-reductase and decrease CYP2C11 in male rat liver. Partial normalization of these enzymes was achieved by gonadotropin or androgen administration to cisplatin-treated male rats [10]. Thus, these findings implicate disruption of the hypothalamic-pituitary-gonadal axis by cisplatin, which, although prolonged, is reversible by hormonal manipulation. Similarly, hepatic microsomal 5α-reductase activity in cyclophosphamide-treated rats was reversed partially by either gonadotropin or the combination of gonadotropin and triiodothyronine [35].

The present study has found that administration of ATRA to male rats increased the function and expression of the hepatic microsomal 5α-reductase at a pretranslational level; a concomitant decline in CYP2C11-mediated steroid hydroxylation also occurred. Thus, a partial biochemical feminization of steroid biotransformation in male rats was elicited. However, complete feminization of 5α-reductase activity was achieved by ATRA administration to gonadectomized animals. It emerges from these studies that ATRA may be a novel positive regulator of the 5α-reductase in rat liver.

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