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DISTINCT TESTICULAR 17-KETOSTEROID REDUCTASES, ONE IN INTERSTITIAL TISSUE AND ONE IN SEMINIFEROUS TUBULES

DIFFERENTIAL MODULATION BY TESTOSTERONE AND METABOLITES OF TESTOSTERONE

EISUKE P. MURONO * and ANITA H. PAYNE

Steroid Research Unit, Departments of Obstetrics and Gynecology and Biological Chemistry, The University of Michigan, Ann Arbor, Mich. 48109 (U.S.A.)

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Summary

The final step in the biosynthesis of testosterone is the reduction of androstenedione, which is catalyzed by the microsomal enzyme 17-ketosteroid reductase. Evidence is presented which suggests that there are two distinct 17-ketosteroid reductases in rat testes, one in interstitial tissue and one in seminiferous tubules. The two enzymes have different pH optima, 5.6 for the one from interstitial tissue and 6.5 for the one from seminiferous tubules. At the optimum pH, a 70-fold difference in K_m values was observed, 17 μM for the interstitial tissue enzyme and 0.25 μM for the enzyme from seminiferous tubules. Testosterone and metabolites of testosterone have very different effects on each of these enzyme activities. The interstitial tissue enzyme activity is inhibited by testosterone and several 5α-reduced metabolites of testosterone and by estrogens. The most potent inhibitor of the steroids investigated was 5α-androstane-3α,17β-diol, followed by 17β-estradiol $\cong$ dihydrotestosterone $>$ testosterone $>$ estrone $>$ estriol. 5α-Androstane-3α,17β-diol and 17β-estradiol were shown to act by competitive inhibition with apparent K_i values of 2.2 and 3.7 μM, respectively. In contrast, it was demonstrated that among the above steroids, only dihydrotestosterone inhibits the 17-ketosteroid reductase activity of seminiferous tubules and this inhibition was only observed at very high concentrations of inhibitor. Testosterone stimulated the 17-ketosteroid reductase activity of seminiferous tubules. 5α-Androstane-3α,17β-diol at low concentrations stim-

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Abbreviations used: 17-ketosteroid reductase 17β-hydroxysteroid:NAD^+ 17-oxidoreductase, EC 1.1.1.64; 3β-hydroxysteroid dehydrogenase-isomerase, 3β-hydroxy-Δ^5-steroid:NAD^+ 3-oxidoreductase, EC 1.1.1.145; HEPES, *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid; Mes, 2[*N*-morphinol]ethane sulfonic acid; Tes, *N*-tris[hydroxymethyl]methyl-2-aminoethane sulfonic acid; Taps, tris[hydroxymethyl]methyl amino sulfonic acid.

ulated the enzyme activity from seminiferous tubules, while it had no effect at high concentrations. The remainder of the steroids tested had no effect on the 17-ketosteroid reductase activity of seminiferous tubules. The difference in response of the two enzyme activities suggests a mechanism for local regulation of testosterone synthesis in each testicular compartment that does not involve directly pituitary gonadotropins.

Introduction

The final step in the biosynthesis of testosterone in the rat testis is the reduction of androstenedione to testosterone. This reaction is catalyzed by the microsomal enzyme 17-ketosteroid reductase (17β-hydroxysteroid:$NADP^+$ 17-oxidoreductase, EC 1.1.1.64) which requires NADPH as hydrogen donor [1]. To date, very little is known about this enzyme. During maturation of the rat testis, 17-ketosteroid reductase is the last enzyme activity to appear and the last to attain adult levels [2]. Attempts at solubilization of the rat testicular microsomal 17-ketosteroid reductase by several physical and chemical procedures were unsuccessful [3]. Oshima and Ochiai [4] utilizing a $10\,000 \times g$ supernatant fraction from rat and human testes as their source of 17-ketosteroid reductase, reported that the addition of testosterone to the incubation medium resulted in stimulation of enzyme activity in both rat and human testes preparations. In a later study, Inano and Tamaoki [5] reported the solubilization and partial purification of 17-ketosteroid reductase from porcine testicular microsomal fractions. They observed inhibition of the 17-ketosteroid reductase activity by the addition of high concentrations of testosterone to the incubation medium.

This study was undertaken to investigate some of the characteristics of the microsomal 17-ketosteroid reductase of rat testes and to examine more thoroughly the effects on this enzyme of testosterone and metabolites of testosterone. During the initial experiments, we observed that reciprocal plots of the initial velocities of the enzyme reaction as a function of substrate concentration resulted in a biphasic curve. Further studies which are presented in this report demonstrated that this biphasic curve was a reflection of two 17-ketosteroid reductases, one localized in interstitial tissue and one localized in seminiferous tubules of rat testes, and that these two enzymic activities differ from each other in their in vitro responses to testosterone and metabolites of testosterone.

Experimental procedures

Materials. [7α-^{3}H]Androstenedione (4.2 Ci/mmol, [1,2,6,7-^{3}H]androstenedione (83 Ci/mmol), [7(*n*)-^{3}H]pregnenolone (18.6 Ci/mmol) were obtained from Amersham/Searle Corp. [4-^{14}C]Testosterone (50.6 Ci/mol) and [4-^{14}C]-progesterone (57.3 Ci/mol) were obtained from New England Nuclear Corp. Each was recrystallized with its respective authentic crystalline steroid to confirm radiochemical purity. Testosterone, androstenedione, progesterone, dihydrotestosterone, 5α-androstane-3β,17β-diol, 17β-estradiol, estrone, NADPH,

NAD^+, HEPES, Mes, Tes, Taps and bovine serum albumin were obtained from Sigma Chemical Co.; 5α-androstane-3α,17β-diol and estriol from Steraloids; and pregnenolone from Schwarz/Mann. All nonradioactive steroids were recrystallized prior to utilization.

Tissue preparation. Mature male rats (70—90 days old) were obtained from Holtzman Company, Madison, Wisc. Rats were killed by decapitation. Testes were quickly removed, decapsulated and homogenized with a teflon-glass Potter-Elvehjem homogenizer in 4 vols. of 0.25 M sucrose containing 5-mM mercaptoethanol, buffered with 0.05 M potassium phosphate, pH 7.4 (Medium A). The homogenate was centrifuged at 500 × *g* for 15 min and resulting supernatant at 10 000 × *g* for 30 min. The 10 000 × *g* supernatant was subjected to centrifugation at 105 000 × *g* for 90 min to obtain a microsomal pellet. The microsomal pellet was resuspended in cold distilled water, lyophilized and stored at —10° C until used for enzyme assays.

Seminiferous tubules and interstitial tissue were separated by the wet dissection method described by Christensen and Mason [6]. The separation was performed in 2.5 mM HEPES buffer, pH 7.4, containing Krebs-Ringer salts and 0.1% glucose (Medium B). The isolated compartments were collected in Medium B containing 5% bovine serum albumin. Tubules were transferred to a 30 ml test tube and allowed to settle by gravity for 5 min. The supernatant, containing free cells, was removed. This procedure was repeated twice with 10 ml of fresh Medium B containing albumin. Isolated tubules were washed 5 additional times with 10 ml of Medium B only. Tubules were then collected on a layer of fine silk cloth and rinsed with 20 ml of Medium B.

Isolated interstitial tissue webs were carefully transferred by fine forceps into a 25 ml beaker containing fresh Medium B. This procedure was repeated twice to remove contaminating germ cells. Interstitial tissue webs were collected on a layer of fine silk cloth as described above for tubules. Isolated interstitial tissue and tubules were homogenized in 4 vols. of Medium A. The homogenates were then subjected to differential centrifugation as described for intact tissue to obtain microsomal fractions. All procedures were performed at 4° C.

Protein was measured by the method of Lowry et al. [7] using crystalline albumin as standard.

Enzyme assays. 17-Ketosteroid reductase activity was determined by measuring the reduction of androstenedione to testosterone. Approximately 1 μCi [^{3}H]androstenedione, plus appropriate amounts of cold androstenedione were dissolved in 0.03 ml dimethylsulfoxide prior to the addition of 0.87 ml of buffer, as indicated for each experiment, containing 0.1 M KCl and 0.1 mM NADPH. The reaction was started by the addition of 0.1 ml appropriate homogenized microsomal preparation equivalent to 0.4 mg protein for intact testis and seminiferous tubules and 0.04 mg protein for interstitial tissue. Incubations were carried out at 37° C for 10 min. The time period and the amounts of protein incubated were based on preliminary studies with each microsomal preparation, which showed that the enzymic reaction was linear under these experimental conditions. A 0.5 ml aliquot was removed at the appropriate time and delivered into a culture tube containing 3 ml 0.1 M NaOH and 15 ml toluene. Each tube contained 25 μg [^{14}C]testosterone (approx. 5000 dpm) for quantitation of the product. The tubes were shaken and placed in a solid CO_2/acetone

bath to freeze the aqueous phase containing the protein. The organic phase which contained the steroids was filtered and dried under air. The residue was dissolved in methanol and submitted to paper chromatography in a Bush B_3 system, heptane/benzene/methanol/H_2O (66 : 34 : 80 : 20, by vol.). The area representing testosterone was identified under ultraviolet light, eluted, and counted in 10 ml scintillation fluid. Testosterone was the only detectable product formed during the incubation. The product isolated in this manner was 96% pure based on recrystallization with authentic crystalline testosterone. All incubations were run at least in duplicate. Zero time controls which were identical to each experimental tube were processed simultaneously with the experimental samples. Enzyme activity (v) is expressed as pmol or nmol testosterone formed per min per mg microsomal protein.

Δ^5-3β-Hydroxysteroid dehydrogenase-isomerase activity was determined by measuring the conversion of pregnenolone to progesterone. Approximately 0.5 μCi [^{3}H]pregnenolone, plus appropriate amounts of cold pregnenolone, were dissolved in 0.03 ml dimethylsulfoxide prior to the addition of 0.87 ml of 0.05 M potassium phosphate buffer, pH 7.4, containing 0.1 mM EDTA and 0.2 mM NAD^+. The reaction was initiated by the addition of 0.1 ml of homogenized microsomal preparation from interstitial tissue or seminiferous tubules equivalent to 0.04 mg and 0.4 mg protein, respectively. Incubations were carried out at 37°C for 10 min. A 0.5 ml aliquot of the incubation mixture was removed and processed as described for the 17-ketosteroid reductase assay, except that 25 μg [^{14}C]progesterone (approx. 5000 dpm) was added for quantitation of the product. The resulting residue was dissolved in methanol and chromatographed on thin layer plates (ITLC-SA, Gelman Instrument Co.) with chloroform/methanol (99.5 : 0.5, v/v). The area representing progesteron was visualized under ultraviolet light, cut out, placed in 10 ml of scintillation fluid and counted. To check the purity of the product, a representative sample was eluted with methanol from the thin layer plate and subjected to recrystallization with authentic crystalline progesterone. The product was 99% pure progesterone.

Results

Enzyme kinetics in microsomal preparation from intact testis. The effect of increasing concentration of androstenedione on the initial velocities of the microsomal 17-ketosteroid reductase from intact testis was analyzed according to Lineweaver-Burk and is illustrated in Fig. 1. The resulting double reciprocal plot was biphasic. This suggested two catalytic sites of different binding affinities for androstenedione, one with an apparent K_m of 1.3 μM and the other with an apparent K_m of 6.3 μM. The inset is the same data analyzed according to Hofstee [8], which likewise gave two distinct slopes with the same respective K_m values.

Δ^5-3β-hydroxysteroid dehydrogenase-isomerase and 17-ketosteroid reductase activities in interstitial tissue and seminiferous tubules. Since the testis has two anatomically distinct compartments, it was possible that the anomalous double reciprocal plot represented two enzymes, one localized in interstitial tissue and one in seminiferous tubules. To answer this question, the 17-ketosteroid reduc-

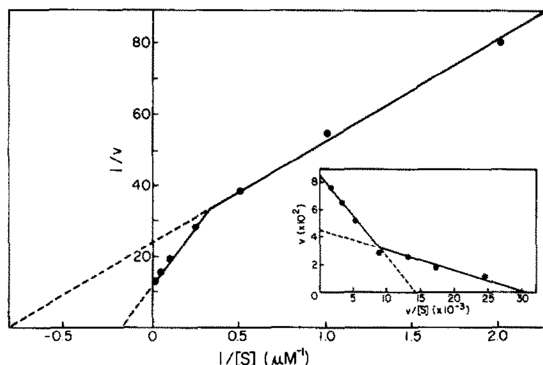

Fig. 1. Representative Lineweaver-Burk plot of the effect of increasing concentration of androstenedione on the rate of testosterone formation by a microsomal preparation from intact rat testes. Microsomal suspension (0.4 mg protein) was incubated with varying concentrations of [^{3}H]androstenedione (1.0—40 μM) for 10 min at 37°C in 0.05 M potassium buffer, pH 6.8, containing 0.1 mM NADPH. Velocity (v) is expressed as nmol testosterone formed per min per mg protein. Inset is the same data plotted according to Hofstee [8].

tase activity was investigated in microsomal fractions from isolated interstitial tissue and from isolated seminiferous tubules. To determine the efficacy of the separation of the compartments, Δ^5-3β-hydroxysteroid dehydrogenase-isomerase activities were determined in the isolated testicular compartments. These enzyme activities have been localized in interstitial tissue of the rat testis [9]. The Δ^5-3β-hydroxysteroid dehydrogenase-isomerase and 17-ketosteroid reductase activities observed in the isolated compartments as well as the ratio of each enzyme activity in interstitial tissue to that in seminiferous tubules is presented in Table I. The ratio of Δ^5-3β-hydroxysteroid dehydrogenase-isomerase activities in interstitial tissue to seminiferous tubules was 797. This indicated that the procedure used to isolate interstitial tissue from seminiferous tubules

TABLE I

17-KETOSTEROID REDUCTASE AND Δ^5-3β-HYDROXYSTEROID DEHYDROGENASE-ISOMERASE ACTIVITIES IN MICROSOMAL FRACTIONS FROM INTERSTITIAL TISSUE AND FROM SEMINIFEROUS TUBULES

Microsomal preparations from interstitial tissue (0.04 mg protein) and from seminiferous tubules (0.4 mg protein) were used in each incubation. Δ^5-3β-Hydroxysteroid dehydrogenase-isomerase activity was determined by measuring the amount of [^{3}H]progesterone formed from 20 μM [^{3}H]pregnenolone in 0.05 M potassium phosphate buffer, pH 7.4, containing 0.2 mM NAD$^+$ during a 10 min incubation at 37°C. 17-ketosteroid reductase activity was determined by the amount of [^{3}H]testosterone formed from 10 μM [^{3}H]androstenedione in 0.05 M potassium phosphate buffer, pH 6.8, containing 0.1 mM NADPH during a 10 min incubation at 37°C.

Enzyme	(pmol/min per mg protein)		Interstitial tissue/seminiferous tubules
	Interstitial tissue	Seminiferous tubules	
17-Ketosteroid reductase	160	3.64	44
Δ^5-3β-Hydroxysteroid dehydrogenase-isomerase	1778	2.23	797

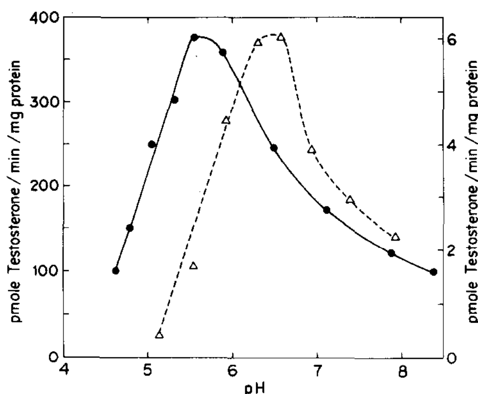

Fig. 2. Effect of pH on the reduction of androstenedione to testosterone. Incubations were carried out for 10 min at 37°C in the appropriate buffer containing 0.1 mM NADPH. The following buffers were used: 0.05 M Mes, pH 4.6—7.0; 0.05 M Tes, pH 7.0—7.9; 0.05 M Taps, pH 7.9—8.4. (a) Microsomal suspension of interstitial tissue (●———●), 0.04 mg protein and 20 μM [^{3}H]androstenedione. (b) Microsomal suspension of seminiferous tubules (△———△), 0.4 mg protein and 10 μM [^{3}H]androstenedione.

yielded tubules essentially free of Leydig cell contamination. The ratio observed for 17-ketosteroid reductase activity in interstitial tissue to that in seminiferous tubules was 44. This lower ratio compared to the ratio for Δ^5-3β-hydroxysteroid dehydrogenase-isomerase suggested that seminiferous tubules contain an intrinsic 17-ketosteroid reductase.

pH optimum for the 17-ketosteroid reductase from interstitial tissue and from seminiferous tubules. The effect of pH on the 17-ketosteroid reductase of interstitial tissue and of seminiferous tubules was investigated. The results are illustrated in Fig. 2. A pH optimum of approximately 5.6 was observed for the interstitial tissue enzyme and pH optimum of approximately 6.5 was observed for the enzyme from seminiferous tubules.

Enzyme kinetics in microsomal preparations from isolated interstitial tissue and from isolated seminiferous tubules at pH optimum. The effect of increasing concentration of androstenedione on the 17-ketosteroid reductase activity was examined in the isolated testicular compartments. The results are illustrated in Figs. 3a and 3b. A single slope was observed for each enzyme preparation with an apparent K_m of 17 μM and a V of 2.5 nmol testosterone/min per mg protein at pH 5.6 for the interstitial tissue enzyme (Fig. 3a) and an apparent K_m of 0.25 μM and a V of 4 pmol testosterone/min per mg protein at pH 6.5 for the tubular enzyme (Fig. 3b).

Effects of testosterone and metabolites of testosterone on the 17-ketosteroid reductase from interstitial tissue and from seminiferous tubules. The effects of testosterone and possible metabolites of testosterone on the 17-ketosteroid reductase activity in isolated interstitial tissue and in isolated seminiferous tubules was investigated. Steroids utilized in addition to testosterone were dihydrotestosterone, 5α-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol, estrone, 17β-estradiol, and estriol. Each of the added steroids was incubated at equimolar substrate concentration and 5 times substrate concentration. The results which are expressed as percent of control are presented in Table II. The

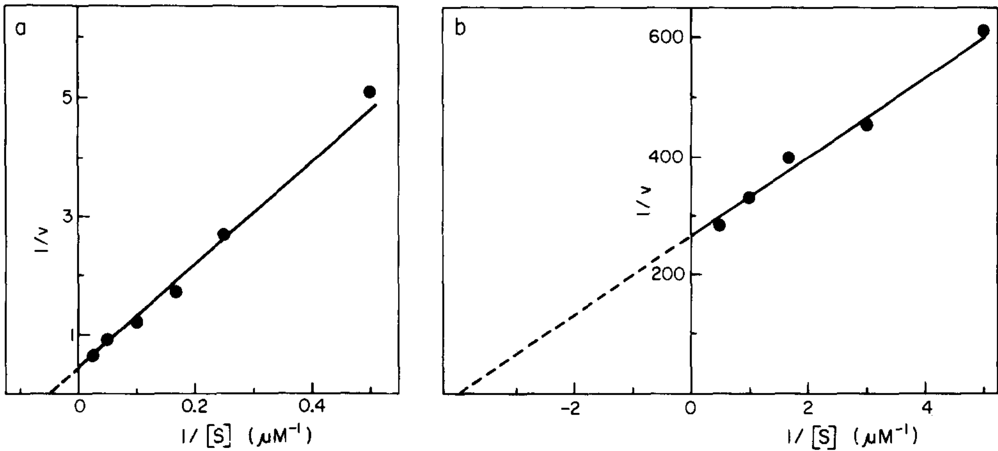

Fig. 3. Representative Lineweaver-Burk plot of the effect of increasing concentration of androstenedione on the rate of testosterone formation by a microsomal preparation. Incubations were carried out for 10 min at 37°C in 0.05 M Mes buffer at the indicated pH, containing 0.1 mM NADPH. Velocity (v) is expressed as nmol testosterone formed per min per mg protein. (a) Interstitial tissue microsomal suspension (0.04 mg protein) was incubated with varying concentrations of [^{3}H]androstenedione (2—40 μM) at pH 5.6. (b) Microsomal suspension of seminiferous tubules (0.4 mg protein) was incubated with varying concentrations of [^{3}H]androstenedione (0.2—2.0 μM) at pH 6.5.

interstitial tissue enzymic activity was inhibited by each of the added steroids. At 5 times substrate concentration the greatest inhibition was observed with 17β-estradiol, 5α-androstane-3α,17β-diol, and dihydrotestosterone followed by testosterone > estrone > estriol. No inhibition by these added steroids was observed on the 17-ketosteroid reductase activity of seminiferous tubules. In contrast, testosterone and 5α-androstane-3α,17β-diol stimulated the 17-ketosteroid reductase activity of seminiferous tubules; at 5 times substrate concentration,

TABLE II

EFFECT OF TESTOSTERONE AND METABOLITES OF TESTOSTERONE ON 17-KETOSTEROID REDUCTASE FROM INTERSTITIAL TISSUE AND FROM SEMINIFEROUS TUBULES

Microsomal preparation from interstitial tissue (0.04 mg protein) and from seminiferous tubules (0.4 mg protein) was incubated with 10 μM or 5 μM [^{3}H]androstenedione, respectively, for 10 min at 37°C in 0.05 M Mes buffer, pH 6.8, containing 0.1 mM NADPH. Steroids were added to the incubation medium as indicated. Values are expressed as percent of control.

Steroids added	Concentration of steroid added (μM)			
	To interstitial tissue		To seminiferous tubules	
	10	50	5	25
None	100	100	100	100
17β-Estradiol	34	5	99	99
Estrone	61	42	104	105
Estriol	76	60	96	98
Testosterone	49	25	137	251
Dihydrotestosterone	38	18	102	93
5α-Androstane-3α, 17β-diol	30	11	124	195

testosterone and 5α-androstane-3α,17β-diol increased enzyme activity to 251 and 195%, respectively. The experiment was repeated with the incubations being carried out at the optimal pH for each enzyme preparation, at 5.6 for the interstitial tissue and at 6.5 for seminiferous tubules. Similar results were obtained. In addition to the steroids listed in Table II, the effect of 5α-androstane -3β,17β-diol was investigated. 5α-Androstane-3β,17β-diol was inhibitory for the interstitial tissue enzyme, but had no effect on the enzyme activity of the seminiferous tubules. This is in contrast to the 5α-androstane-3α,17β-diol, which had a stimulatory effect on the tubular enzyme activity at either pH.

To ascertain that these steroids had no inhibitory effect on the tubular 17-ketosteroid reductase activity, we repeated the above experiment with a lower substrate concentration and a higher concentration of added steroids. The latter were included in the incubation medium at 50 and 500 times substrate concentration. The results are presented in Table III. Under these conditions, we still did not observe an inhibitory effect on the activity of the tubular 17-ketosteroid reductase with 17β-estradiol or with 5α-androstane-3β,17β-diol. However, dihydrostestosterone did inhibit the enzyme activity at these low substrate and high dihydrotestosterone concentrations. Testosterone, as demonstrated earlier, again stimulated the tubular enzyme activity. However, with 5α-androstane-3α, 17β-diol we observed a lesser degree of stimulation at 50 times substrate concentration, and no inhibition at 500 times substrate concentration.

Type of inhibition of the interstitial tissue 17-ketosteroid reductase by 5α-androstane-3α,17β-diol and 17β-estradiol. Increasing fixed concentrations of 5α-androstane-3α,17β-diol caused changes in slopes, but not the intercept in double reciprocal plots as a function of 1/[androstenedione] (Fig. 4a). This is consistent with 5α-androstane-3α,17β-diol acting as a competitive inhibitor of androstenedione for the 17-ketosteroid reductase. The apparent K_i of 2.2 μM for 5α-androstane-3α,17β-diol was determined from a replot of the slopes as a function of increasing concentrations of the inhibitor (Fig. 4a, inset). A comparable experiment was carried out with increasing concentrations of 17β-estradiol. As illustrated in Fig. 4b, 17β-estradiol was also found to be a competitive inhibitor with an apparent K_i of 3.7 μM.

TABLE III

EFFECT OF HIGH CONCENTRATIONS OF TESTOSTERONE AND METABOLITES OF TESTOSTERONE ON 17-KETOSTEROID REDUCTASE FROM SEMINIFEROUS TUBULES

Microsomal preparation from seminiferous tubules (0.4 mg protein) was incubated with 0.2 μM [^{3}H]-androstenedione and added steroid as indicated for 10 min at 37°C in 0.05 M Mes buffer, pH 6.5, containing 0.1 mM NADPH. Values are expressed as per cent of control.

Steroid added	Concentration of steroid added	
	10 μM	100 μM
None	100	100
17-Estradiol	106	102
Testosterone	130	193
Dihytestosterone	79	43
5α-Androstane-3α, 17β-diol	127	83
5α-Androstane-3β, 17β-diol	98	93

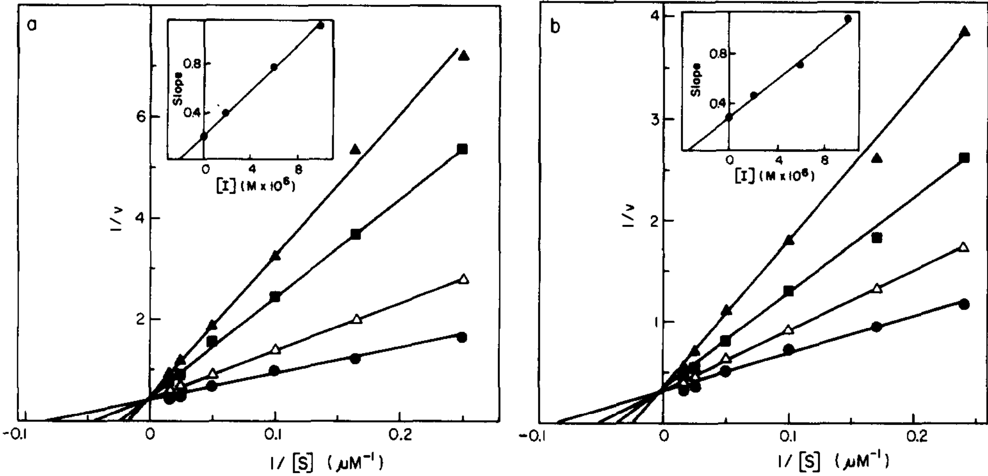

Fig. 4. Double reciprocal plots of velocity vs. concentration of androstenedione at various fixed concentrations of 5α-androstane-3α,17β-diol or 17β-estradiol by a microsomal preparation from interstitial tissue. (a) Interstitial tissue microsomal suspension (0.04 mg protein) was incubated for 10 min at 37°C in 0.05 M Mes buffer, pH 5.6, containing 0.1 mM NADPH with increasing concentrations of [^{3}H]androstenedione (4—60 μM) and the following fixed concentrations of 5 α-androstane-3α,17β-diol: 0, (●——●); 2 μM, (△——△); 6 μM, (■——■); 10 μM, (▲——▲). Velocity (v) is expressed as nmol testosterone formed per min per mg protein. Inset is a plot of the slopes as a function of increasing concentrations of 5α-androstane-3α,17β-diol. (b). Same conditions as in (a). Concentrations of 17β-estradiol: 0, (●——●); 2 μM, (△——△); 6 μM, (■——■); 10 μM, (▲——▲). Inset is a plot of the slopes as a function of increasing concentrations of 17β-estradiol.

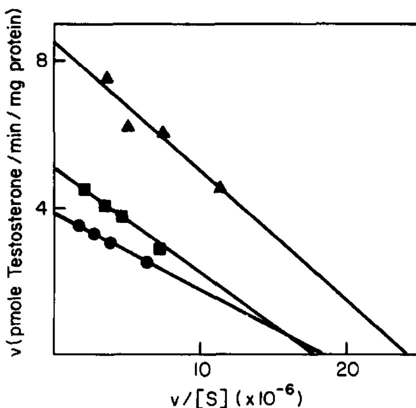

Fig. 5. Hofstee plot of the effect of increasing concentrations of androstenedione on the rate of testosterone formation at various fixed concentrations of testrosterone by a microsomal preparation from seminiferous tubules. Microsomal suspension (0.4 mg protein) was incubated for 10 min at 37°C in 0.05 MMes buffer, pH 6.5, containing 0.1 mM NADPH with increasing concentrations of [^{3}H]androstenedione (0.4—2 μM) and fixed concentrations of testosterone: 0, 1 and 2 μM, (●——●); 5 μM, (■——■); 25 μM, (▲——▲). Velocity (v) is expressed as pmol testosterone formed per min per mg protein. The Y intercepts represent V and the slope $-K_m$.

Stimulation of 17-ketosteroid reductase activity of seminiferous tubules by testosterone. Enzyme activity was determined with increasing concentrations of substrate and increasing fixed concentrations of testosterone. The results were plotted according to Hofstee and are presented in Fig. 5. No effect of testosterone was observed at concentrations of 1 or 2 μM, while concentrations of 5 and 25 μM stimulated the 17-ketosteroid reductase activity. The stimulation was proportional to the concentration of testosterone added. The principal effect of testosterone was to increase V.

Discussion

The present study suggests the presence of two distinct 17-ketosteroid reductases in microsomal preparations of rat testes, one in interstitial tissue and one in seminiferous tubules. Evidence for distinct 17-ketosteroid reductases in each testicular compartment is based on the following observations: (1) different K_m values, (2) different pH optima, (3) diverse effects of a variety of steroid metabolites on the two enzyme activities.

At the pH optimum of each enzyme, a 70-fold difference was observed between the K_m value of the interstitial tissue enzyme activity and the K_m value of the tubular enzyme activity. In earlier studies from this laboratory, we demonstrated intrinsic 17-ketosteroid reductase activity with androstenedione [10] and dehydroepiandrosterone [11] as substrates in isolated seminiferous tubules from human testes. In those studies, no attempt was made to characterize the 17-ketosteroid reductase of seminiferous tubules. Richards and Neville [12] observed 17-ketosteroid reductase activity in isolated seminiferous tubules from rat testes with dehydroepiandrosterone as substrate. Oshima et al. [13] recently reported on kinetic studies of the 17-ketosteroid reductase in cell free homogenates from intact human testes. With dehydroepiandrosterone as substrate, they observed two distinct slopes in double reciprocal plots of their data. This is consistent with our finding of 17-ketosteroid reductase activity in isolated seminiferous tubules and suggests that the human testis also contains two distinct 17-ketosteroid reductases, one in each testicular compartment. Although the K_m of the rat interstitial tissue enzyme is much higher than the K_m of the tubular enzyme, the concentration of the enzyme in interstitial tissue is markedly greater, as indicated by a much higher V. At the optimum pH, V of the 17-ketosteroid reductase in interstitial tissue was 2.5 nmol testosterone/min per mg protein, while that in seminiferous tubules was 4 pmol testosterone/min per mg protein. The relative contribution of the tubular enzyme to the synthesis of testosterone in the intact animal may be greater than the V value would suggest, since seminiferous tubules comprise about 95% of rat testicular tissue [14].

The inhibitory effect of the interstitial tissue 17-ketosteroid reductase by several metabolites of testosterone may play an important role in local modulation of testosterone synthesis that does not involve directly pituitary gonadotropins. Tcholakian et al. [15] recently reported that a single subcutaneous injection of estradiol benzoate to adult male rats markedly reduced testicular and serum testosterone levels without having an effect on the concentration of plasma or pituitary luteinizing hormone. Both 17β-estradiol and 5α-androstane-

3α,17β-diol were shown to act by competitive inhibition and exhibited K_i values considerably lower than the apparent K_m for androstenedione. We recently reported that the major site of estradiol synthesis in the human testis is the interstitial tissue [16]. Estradiol synthesized within the interstitial tissue may thus act on the 17-ketosteroid reductase by feedback inhibition. The major site of 5α-androstane-3α,17β-diol synthesis in the rat [17] and in man [18] is the seminiferous tubules. It is not known whether 5α-androstane-3α,17β-diol is transported or can diffuse from the tubules to the interstitial tissue where it could act to inhibit the 17-ketosteroid reductase and thus modulate testosterone synthesis in the Leydig cell. Stimulation of 17-ketosteroid reductase by testosterone has been reported in rat testes and human testes [4,13], while an inhibition of this enzyme by testosterone was observed with a partially purified 17-ketosteroid reductase from porcine testes [5]. In the present study, only the tubular 17-ketosteroid reductase activity was stimulated by testosterone, while the interstitial tissue 17-ketosteroid reductase activity was inhibited by testosterone.

5α-androstane-3α,17β-diol, which acts as a potent inhibitor of the interstitial tissue 17-ketosteroid reductase activity, stimulates this enzyme activity in seminiferous tubules. The stimulatory effect is only seen at the lower concentrations while a higher concentration (0.1 mM) appears to have no effect. The mechanism by which testosterone and low concentrations of 5α-androstane-3α,-17β-diol stimulate the 17-ketosteroid reductase activity of seminiferous tubules requires additional studies. However, stimulation by testosterone and by low concentrations of 5α-androstane-3α,17β-diol appears to be very specific for these steroids, since this effect was not observed with two structurally stimilar androgens, dihydrotestosterone or 5α-androstane-3β,17β-diol.

The demonstration in the present study of two distinct 17-ketosteroid reductases and the differential effect of steroid metabolites on each of these enzyme activities suggests mechanisms by which testosterone synthesis may be regulated locally in interstitial tissue and in seminiferous tubules; a mechanism that does not directly involve pituitary hormones.

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