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INHIBITION OF PROSTATIC 5α-REDUCTASE AND 3α-HYDROXYSTEROID DEHYDROGENASE BY TWO ANTIANDROGENS

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SUMMARY

This study is directed towards the evaluation of synthetic compounds having the capacity to inhibit the metabolism of androgens within the rat ventral prostate gland. Two antiandrogens, L586.659 (A) and L598.229 (B) were investigated to determine their effect on the above enzyme activities. Compound A, possessing a chemical structure similar to testosterone, was found to be a potent inhibitor of 5α-reductase activity; the inhibition was of the competitive type and the K_i obtained was 5×10^{-8} M. Compound B, with a ring A structure identical to dihydrotestosterone, was found to be a strong competitive inhibitor of 3α-hydroxysteroid dehydrogenase with a K_i of 2.5×10^{-6} M.

INTRODUCTION

In several target organs, such as the prostate, seminal vesicles and sebaceous glands, it has been demonstrated that androgen action is mediated by metabolites of testosterone (T), principally the 5α-reduced metabolite dihydrotestosterone (DHT) [1–8]. The importance of DHT became evident when it was found to be the principal steroid bound to the cytosol and nucleus of the prostatic cell [9–12]. Further metabolism of DHT to the androstanediols (3α or 3β-diols) should also be considered as an important metabolic step, at least in the prostate, since Baulieu *et al.* have demonstrated a biological effect of these compounds on the prostate in organ cultures [13]. It has been recently shown that 3α,17β-diol is most probably an important androgen in the dog prostate [14–15] and recent data suggest that 3α,17α-diol is also important for the activity of this tissue [16]. Therefore, the prostatic enzymes, 5α-reductase and hydroxysteroid dehydrogenase are important determinants of the action and concentration of androgens in such target organs.

Early studies of the reaction catalyzed by 5α-reductase indicate that NADPH is the cofactor required as hydrogen donor [1, 17–21] and that the transformation is thermodynamically irreversible [22]. Subcellular fractionation indicates that 5α-reductase is concentrated in the nuclear and the microsomal fractions [18–21, 23] and the properties of the enzyme are the same in both types of organelle [24]. Moore and Wilson [25] and Nozu and Tamaoki [26] reported that 5α-reductase in rat prostatic nuclei is located in the membrane fraction. Optimal pH is obtained near neutral values [19–21, 24] and optimal temperature is 37°C [20, 24].

This knowledge of the mechanism of action of androgens has led to the use of antiandrogens in many clinical disorders. These compounds exert their action at two different levels: either they impair the formation of DHT or the formation and translocation of the DHT–receptor complex. The antiandrogen, cyproterone acetate has been shown to have an effect on the DHT–receptor complex [27–31]. Fang and Liao [31] also observed a displacement of DHT from the nuclear receptor by cyproterone acetate. Flutamide [32–33], Spirolactone [34–35], compound R2956 [36] and BOMT [37] (4′-nitro-3′-trifluoromethylisobutyrylanilide) also appear to influence the formation of the DHT–receptor complex. However, Tan *et al.* [38] have recently demonstrated an inhibition of DHT formation in the presence of cyproterone acetate and flutamide. High concentrations (10^{-6}–10^{-5} M) of several steroids such as progesterone [39–43], 17-hydroxyprogesterone [42–43], estrogens [41, 43–46], desoxycorticosterone, androstenedione [42] and compound R4414 [47] can inhibit the formation of DHT. Voigt and Hsia also report an inhibition of 5α-reductase activity by 4-androsten-3-one-17β-carboxylic acid [48]; this compound blocks the androgen-dependent enlargement of the hamster costo-vertebral organ [49].

3α-Hydroxysteroid dehydrogenase is another important enzyme controlling the intracellular concentration of DHT in the ventral prostate [50–52]. This enzyme has also been detected in various human and rat tissues, eg. lung [53], skin [54–55] and seminal vesicles [6]; it is located in the soluble fraction of prostatic cells [1, 50, 52], has an optimal pH which is also near neutral values and it prefers NADPH as cofactor [50, 52]. Many 4-ene-3-ketosteroids can

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serve as competitive inhibitors of this enzyme although it would appear that 5α or 5β-reduction must occur before they function as effective inhibitors [52]. Medroxyprogesterone acetate [43, 45] is also a good inhibitor of the enzyme.

It is evident, therefore, that selective inhibitors of 5α-reductase and of 3α-hydroxysteroid dehydrogenase could be important tools to promote the basic understanding of androgen function. The present investigation concerns the *in vitro* effect of two antiandrogens on prostatic 5α-reductase and 3α-hydroxysteroid dehydrogenase. The kinetics of the enzyme reactions were measured in the presence of each compound and the nature of the inhibition was determined.

MATERIALS AND METHODS

Reagents. Solvents used were of analytical grade and ether was purified on aluminum oxide columns (Woelm neutral, Activity 1, Waters Associates Inc., Framingham, MA). $NADP^+$, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were obtained from Sigma; solutions of these reagents in buffer were prepared immediately before use. The activity of the glucose-6-phosphate dehydrogenase was verified according to the method of Glock and McLean [56].

[4-^{14}C]-Testosterone (56.1 and 57.5 mCi/mmol), [1,2-^{3}H]-dihydrotestosterone (44 and 43.4 Ci/mmol) and [1,2-^{3}H]-5α-androstane-3α,17β-diol (50.0 and 41.7 Ci/mmol) were purchased from New England Nuclear, Boston, MA. These steroids were purified by chromatography and the radiochemical purity was verified by crystallization with authentic carrier prior to use. The purified tracers were dissolved in ethanol and stored at 4°C. Unlabelled DHT and 3α-diol (Mann Research Labs, New York, NY) were crystallized from methanol–water and used as carrier steroids. The antiandrogens L586.659 (A) and L598.229 (B), whose structures are presented in Fig. 5, were obtained from Merck and Co., Rahway, NJ. Solutions of these compounds were prepared in purified ethanol.

Preparation of tissue. Wistar rats weighing between 200–250 g were sacrificed and the ventral prostates were immediately excised. The prostates were washed with ice-cold Tris–HCl buffer (Tris 0.05 M, EDTA 1.5 mM, mercaptoethanol 2 mM, pH 7.4), minced with scissors and homogenized at 5°C in ten vol. of buffer using a Potter–Elvejem homogenizer. The homogenate was filtered through four layers of cheese cloth and centrifuged at 900 *g* for ten min in a Sorvall RC2-B centrifuge to obtain the crude nuclear fraction. The pellets were washed and the nuclear fractions were, in some instances, purified further by centrifugation on a sucrose gradient as described by Widnell and Tata [57]. The 900 *g* supernatant was centrifuged at 105,000 *g* for 60 min in a Beckman model L5-65 centrifuge to obtain the soluble fraction. Subcellular fractions were stored at −20°C prior to use.

Protein was determined by the method of Lowry *et al.* [58] with bovine serum albumin as the reference protein.

Enzyme assay. The substrates, [4-^{14}C]-testosterone or [1,2-^{3}H]-dihydrotestosterone, were used at concentrations ranging from 10^{-8}–5×10^{-6} M. The antiandrogens were added to different concentrations of the substrate. The NADPH generating system consisted of $NADP^+$ (10^{-4} M), glucose-6-phosphate (2.5×10^{-3} M) and glucose-6-phosphate dehydrogenase (10^{-3} g/ml or 1.8 U). To initiate the reaction, the nuclear (5α-reductase activity) or soluble (3α-hydroxysteroid dehydrogenase activity) fraction was added to the incubation medium to a final vol. of 5 ml. Incubations were of one h duration at 37°C in a Dubnoff Metabolic Incubator (Precision Scientific Co., Chicago, IL). Incubations were terminated by the addition of one vol. of ether; carrier DHT (200 μg) or 3α-diol (300 μg) was added to each tube in order to facilitate their detection and to account for procedural losses. Known amounts of [1,2-^{3}H]-DHT were also added where [4-^{14}C]-testosterone served as substrate.

Three extractions (v/v) were performed and the ether extracts were combined, dried over 4–5 g of anhydrous sodium sulfate and evaporated to dryness. The residue was then purified by paper or thin layer chromatography using the following systems: (A), Ligroin–methanol–water (100:90:10, by vol.) at ambient temperature for 9 h; support: paper. (B), Ligroin–methanol–water (100:90:10, by vol.) at 4°C; support: paper. (C), Benzene–ethyl acetate (1:1, v/v); 4 h on alumina. (D), Benzene–ether (2:1, v/v); 4 h on silica gel. (E), Benzene–ethanol (95:5, v/v); 6 h on alumina. The 20 cm × 20 cm plates were precoated with alumina (Eastman Chromatogram Sheet 6062) or silica gel (Eastman Chromatograph Sheet 6061).

Radioactive products were located by scanning papers or t.l.c. plates with a Packard Radiochromatogram scanner, model 7201. Nonradioactive steroids were located as follows: testosterone and androstenedione by ultraviolet absorption; dihydrotestosterone, androsterone and etiocholanolone with the standard Zimmermann reaction (potassium hydroxide 15%, *m*-dinitrobenzene 2%); 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol with an alcoholic solution of phosphomolybdic acid.

Acetylation was performed by adding a mixture of pyridine–acetic anhydride (2:1, v/v) to the dried extract and the reaction was left to proceed overnight at room temperature. The excess reagents were evaporated under a stream of nitrogen. Following the addition of mg amounts of authentic steroid, each purified metabolite was recrystallized from methanol–water to constant ^{3}H/^{14}C ratio or to constant specific activities. Radioactivity was assayed using a Packard liquid scintillation spectrometer, model 3375. Maximal counting efficiency was 68% for ^{14}C and 44% for ^{3}H using a dual label discriminator setting.

Table 1. Effect of antiandrogen L586.659 (A) on 5α-reductase activity

Concentration of antiandrogen A	Relative transformation of T to DHT (%)		
	Experiment 1	Experiment 2	Mean
Control	100.0	100.0	100.0
5×10^{-6} M	21.8	21.2	21.5
1×10^{-5} M	13.8	13.1	13.5
2.5×10^{-5} M	8.6	10.5	9.6

Isolation, purification and quantitative determination of DHT and 5α-androstane-3α,17β-diol. The residue from the ether extract was chromatographed in system A. The area corresponding to the R_F of DHT was eluted from the paper, the solvent was evaporated to dryness, and the residue was rechromatographed in system C and in system D. DHT was then crystallized to constant $^3H/^{14}C$ ratios.

To isolate the diols, the residue was chromatographed on thin layer in system E and then on paper in system A. The product migrating with authentic 3α-diol was crystallized from methanol–water until constant S.A. was achieved in the crystals and in the mother liquor. Recovery of the 3α-diol was calculated after the last chromatographic step by gas–liquid chromatography (Packard gas chromatograph, model 871; support: Chromosorb W; stationary phase: 3% SE-30, Chromatographic Specialities, Brockville, Ontario; carrier gas: nitrogen; flow rate: 40 cc/min; temperature of the column: 252°C and pressure: 10 pounds/square inch). Recovery of carrier 3α-diol was calculated from a standard curve. Final concentrations of the purified metabolites are expressed either in mμmol of product formed/min/mg protein or as a percentage of transformation of the substrate to a given metabolite.

Antiandrogens used for the enzymatic assays. Compound L586.659 (A) {2',3'-tetrahydrofuran-2' → 17-(4-ene-androsten-3-one)} has a ring A structure identical to ring A of testosterone. Concentrations of 2.5×10^{-7} M–2.5×10^{-5} M were used to determine its effect on 5α-reductase activity.

Compound L598.229 (B) {2',3'-tetrahydrofuran-2' → 17(5α-androstan-3-one)} has a ring A structure identical to ring A of DHT. It was investigated for its effect on 5α-reductase and 3α-hydroxysteroid dehydrogenase activities at concentrations ranging from 5×10^{-7} M–2.5×10^{-5} M.

RESULTS

5α-Reductase activity

For the enzymatic assays, the incubation times and the enzyme concentrations were chosen in the linear portion of the curves. The effects of antiandrogen A on 5α-reductase are presented in Table 1. Two experiments were performed and the mean percentage of transformation was calculated for each substrate concentration. A strong inhibition of enzyme activity was obtained with the three concentrations of compound A tested. To study the type of inhibition produced by compound A, lower concentrations of this inhibitor were used eg. 2.5 and 5.0×10^{-7} M at various concentrations of the substrate. The results of this experiment (Table 2) and the corresponding Lineweaver–Burk plot (Fig. 1) clearly indicate a competitive type of inhibition. A K_i value of 5×10^{-8} M was obtained by plotting the reciprocal of V vs. the concentration of the inhibitor (Fig. 2).

When the antiandrogen B was tested for its effect on 5α-reductase activity, no significant inhibition was obtained with the three different concentrations (Table 3).

3α-Hydroxysteroid dehydrogenase activity

The results of the enzymatic assays with antiandrogen B are presented in Table 4. At concentration B_1 (0.5×10^{-6} M) a mean inhibition of 15% was obtained, for concentrations B_2 (5×10^{-6} M) and B_3 (10^{-5} M) the percentages of inhibition were 62% and 76% respectively. These results were plotted graphically according to Lineweaver–Burk as illustrated in Fig. 3. The inhibition was also of the competitive type (Fig. 3). A constant V_{max} was observed and the K_m value varies with the concentration of the antiandrogen. To obtain the inhibition constant, K_i, the reciprocal of the rate of formation of 3α-diol vs the

Table 2. Effect of antiandrogen L586.659 (A) on 5α-reductase activity

Concentration of substrate (M)	DHT Formed/min/mg protein (mμmol × 10^{-3})		
	Control	(A) 2.5×10^{-7} M	(A) 5×10^{-7} M
0.5×10^{-7}	1.93	0.32	0.21
1.1×10^{-7}	2.83	0.70	0.37
5.4×10^{-7}	6.36	3.59	1.80
1.1×10^{-6}	9.57	5.60	3.13
3.3×10^{-6}	12.90	7.73	6.15
5.4×10^{-6}	13.80	—	7.92

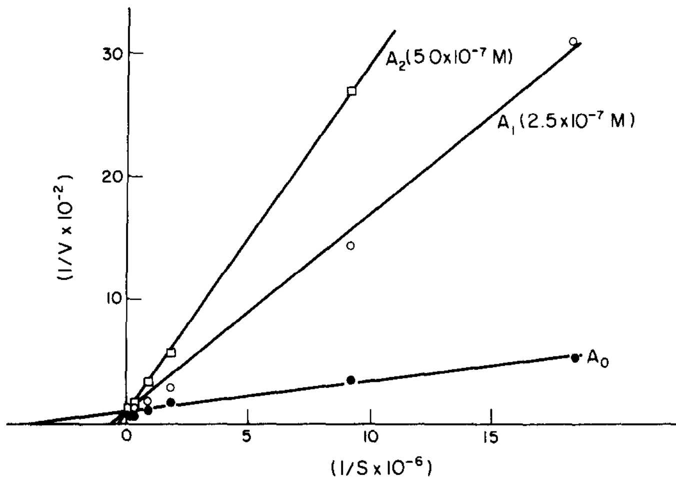

Fig. 1. Competitive inhibition by product L586.659 (A) towards 5α-reductase is illustrated with Lineweaver–Burk plots. The nuclear fraction of rat ventral prostate was incubated under standard conditions. Velocity of the reaction is expressed in mμmol of DHT formed/min/mg prot. and (S) represents the molar concentration of the substrate T. ●——● Enzymatic assay without antiandrogen A (A_0) ○——○ Enzymatic assay with antiandrogen A 2.5×10^{-7} M (A_1) □——□ Enzymatic assay with antiandrogen A 5×10^{-7} M (A_2)

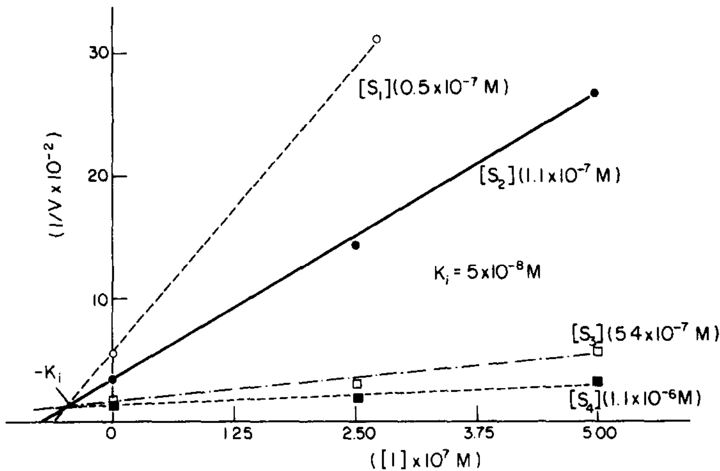

Fig. 2. Determination of K_i for antiandrogen L586.659 (A). Velocity of the reaction is expressed in mμmol of DHT formed/min/mg protein and (I) is the molar concentration of the inhibitor. ○——○ Concentration of T 0.5×10^{-7} M (S_1) ●——● Concentration of T 1.1×10^{-7} M (S_2) □——□ Concentration of T 5.4×10^{-7} M (S_3) ■-----■ Concentration of T 1.1×10^{-6} M (S_4)

concentrations of the inhibitor was plotted as illustrated in Fig. 4. The K_i value, as calculated from Fig. 4, is 2.5×10^{-6} M.

DISCUSSION

DHT is a mediator of androgen action within certain target tissues. It has been postulated that abnormal DHT formation or accumulation in the prostate is involved in the pathogenesis of benign prostatic hypertrophy in man and the dog [59–60], and more recently that 3α-diol could be an important androgen mediator in the dog prostate [14–15]. These findings illustrate that the use of pharmacologic agents to inhibit specifically the formation of DHT or 3α-diol could have useful clinical applications.

Table 3. Effect of antiandrogen L598.229 (B) on 5α-reductase activity

Concentration of antiandrogen B	Relative transformation of T to DHT (%)		
	Experiment 1	Experiment 2	Mean
Control	100.0	100.0	100.0
5×10^{-6} M	112.0	90.3	101.0
1×10^{-5} M	94.2	98.3	96.3
2.5×10^{-5} M	99.4	90.6	95.0

Table 4. Effect of antiandrogen L598.229 (B) on 3α-Hydroxysteroid dehydrogenase activity

Concentration of antiandrogen B	Relative transformation of DHT to 5α-androstane-3α,17β-diol (%)		
	Experiment 1	Experiment 2	Mean
Control	100.0	100.0	100.0
0.5×10^{-6} M	76.7	94.2	85.5
5.0×10^{-6} M	38.0	37.1	37.6
1×10^{-5} M	20.5	28.2	24.4

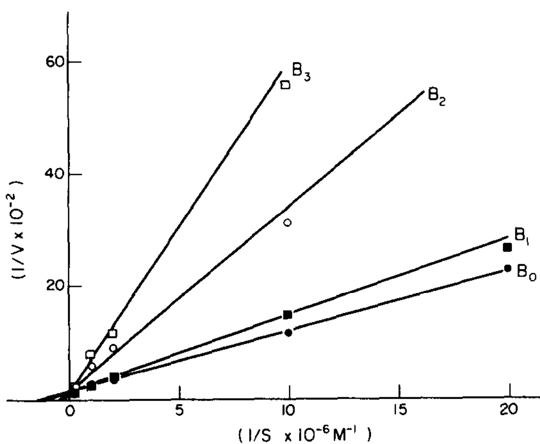

Fig. 3. Competitive inhibition by product L598.229 (B) towards 3α-hydroxysteroid dehydrogenase is illustrated with Lineweaver–Burk plots. The soluble fraction of rat ventral prostate was incubated under standard conditions. Velocity of the reaction is expressed in mμmol of 5α-androstane-3α,17β-diol formed/min/mg protein and (S) represents the molar concentration of the substrate DHT. ●——● Enzymatic assay without antiandrogen B (B_0) ■——■ Enzymatic assay with antiandrogen B 0.5×10^{-6} M (B_1) ○——○ Enzymatic assay with antiandrogen B 5×10^{-6} M (B_2) □——□ Enzymatic assay with antiandrogen B 10×10^{-6} M (B_3)

Several compounds have been tested for antiandrogenic activity and a recent review by Mainwaring [61] summarizes the effects of these compounds. Most antiandrogens impair the formation and/or the translocation of the DHT-receptor complex [62]. Saturation analysis, as described by Shain and Boesel, has allowed the determination of affinity constants [63]. Few androgen analogs have been reported to inhibit the transformation of T to DHT and the existing compounds have a rather low affinity for the enzyme. In order to achieve a complete inhibition of the metabolism of androgens, one must utilize extremely high dosages of these antiandrogens.

In our studies on the physiological action of androgens, efforts were directed toward the search for antiandrogens with high potency and specificity. Compound A (L586.658), one of the two newly synthesized steroids under study is, *in vitro*, the most potent inhibitor of 5α-reductase with a K_i of 5×10^{-8} M. The K_m of the enzyme is of the order of 5×10^{-7} M. Thus, compound A has a ten-fold greater affinity for the enzyme than the natural substrate, testosterone. Since ring A of L586.659 is identical to that of testosterone, this would explain the competitive type of inhibition observed. With regard to specificity, compound A does not, *per se*, inhibit

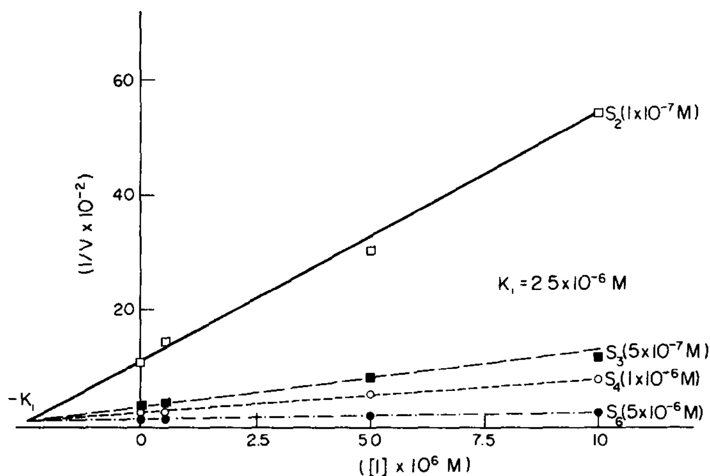

Fig. 4. Determination of K_i for antiandrogen L598.229 (B). Velocity of the reaction is expressed in mμmol of 5α-androstane-3α,17β-diol formed/min/mg protein and (I) represents the molar concentration of the inhibitor. □——□ Concentration of T 1×10^{-7} M (S_2) ■——■ Concentration of T 5×10^{-7} M (S_3) ○——○ Concentration of T 1×10^{-6} M (S_4) ●——● Concentration of T 5×10^{-6} M (S_6)

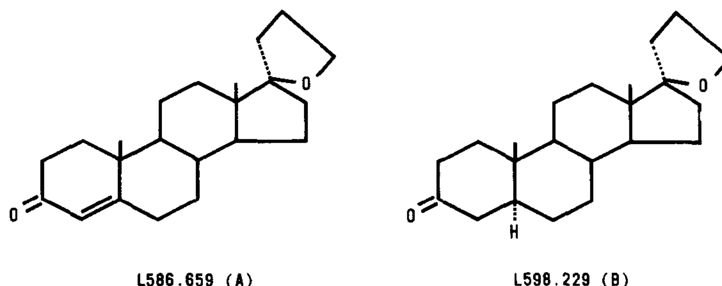

Fig. 5. Structures of antiandrogens A and B.

3α-hydroxysteroid dehydrogenase. However, this compound is also a substrate for 5α-reductase (unpublished results) and it's 5α-reduced metabolite, antiandrogen B in this study, is a potent and specific inhibitor of 3α-hydroxysteroid dehydrogenase (K_i of 2.5×10^{-6} M).

In preliminary experiments (unpublished data), *in vivo*, these compounds exhibited various biological effects depending on the assay system used. When administered to castrated rats, Compound A significantly ($P < 0.01$) inhibited the effect of injected testosterone and dihydrotestosterone (as enanthate esters) on the weight of the ventral prostate. Furthermore, in the chick comb assay this compound significantly inhibits testosterone-induced stimulation. On the other hand, in mature intact gerbils it has no effect on the size and weight of the sebaceous glands.

Compound B, following subcutaneous administration, also significantly decreases ($P < 0.05$) the effect of DHT, but not of testosterone, on the weight of the ventral prostate of the castrated rat. Further studies are warranted on the metabolism of these antiandrogens in intact and castrated animals in order to explain these results.

As Johansson [64] and Johansson and Niemi [65] have observed, the metabolism of testosterone proceeds at a very high rate in cultured prostatic cells; under such conditions, any given androgenic effect cannot be specifically assigned to a particular androgen. One of the ultimate aims of this study is to use antiandrogens to specifically block enzymatic pathways responsible for androgen metabolism, in order to rigorously identify the specific metabolic effect of testosterone, dihydrotestosterone and the 3α and 3β-diols. Ideally, such analogs should be potent and specific, as observed for compounds A and B; they should also be active on whole cell preparations and they should not displace natural androgens from their cellular receptors. It is not yet known whether these two analogs fulfill all of these criteria; studies to evaluate this possibility are currently in progress.

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REFERENCES

1. Bruchovsky N. and Wilson J. D.: *J. biol. Chem.* **243** (1968) 2012–2021.
2. Kowarski A., Shalf J. and Migeon C. J.: *J. biol. Chem.* **244** (1969) 5269–5272.
3. Giorgi E. P., Stewart J. C., Grant J. K. and Scott R.: *Biochem. J.* **123** (1971) 41–55.
4. Becker H., Kaufmann J., Klosterhalfen H. and Voigt K. D.: *Acta endocr., Copenh.* **71** (1972) 589–599.
5. Harper M. E., Pike A., Peeling W. B. and Griffiths K.: *J. Endocr.* **60** (1974) 117–125.
6. Buric L., Becker H., Peterson C. and Voigt K. D.: *Acta endocr., Copenh.* **69** (1972) 153–164.
7. Robel P., Corpéchot C. and Baulieu E. E.: *FEBS Lett.* **33** (1973) 218–220.
8. Ebling F. J., Ebling E., McCofferey V. *J. Endocr.* **51** (1971) 181–190.
9. Bruchovsky N. and Wilson J. D. *J. biol. Chem.* **243** (1968) 5953–5960.
10. Anderson K. M. and Liao S. *Nature, Lond.* **219** (1968) 278–279.
11. Mainwaring W. I. P.: *J. Endocr.* 44 (1969) 323–333.
12. Krieg M., Horst H.-J. and Sterba M.-L. *J. Endocr.* **64** (1975) 529–538.
13. Baulieu E. E., Lasnitzki E. and Robel P.: *Nature* **219** (1968) 1155–1156.
14. Walsh P. C. and Wilson J. D.: *J. clin. Invest.* **57** (1976) 1093–1097.
15. Jacobi G. H. and Wilson J. D.: *Endocrinology* **99** (1976) 602–610.
16. Evans C. R. and Pierrepoint C. G.: *J. Endocr.* **70** (1976) 31–37.
17. Shimazaki J., Kurihara H., Ito Y. and Shida K. Gunma.: *J. Med. Sci.* **15** (1965) 326–333.
18. Mainwaring W. I. P.: *Biochem. biophys. Res. Commun.* **40** (1970) 192–198.
19. Roy A. B.: *Biochimie* **53** (1971) 1031–1040.
20. Frederiksen D. W. and Wilson J. D.: *J. biol. Chem.* **246** (1971) 2584–2593.
21. Shimazaki J., Kurihara H., Ito Y. and Shida K.: *Endocr. Jap.* **20** (1973) 489–496.
22. McGuire J. S. Jr., Hollis V. W. Jr. and Tomkins G. M.: *J. biol. Chem.* **235** (1960) 3112–3117.
23. Nozu K. and Tamaoki B.: *Acta endocr., Copenh.* **73** (1973) 585–598.
24. Nozu K. and Tamaoki B.: *Acta endocr., Copenh.* **76** (1974) 608–624.
25. Moore R. J. and Wilson J. D.: *J. biol. Chem.* **247** (1972) 958–967.
26. Nozu K. and Tamaoki B.: *Biochim. biophys. Acta* **348** (1974) 321–333.
27. Hansson V. and Tveter K. J.: *Acta endocr., Copenh.* **68** (1971) 69–78.
28. Geller J. and McCoy K.: *Acta endocr., Copenh.* **75** (1974) 385–397.
29. Scot Skinner R. W., Pozderac R. V., Counsell R. E. and Weinhold P. A.: *Steroids* **25** (1975) 189–202.
30. Szalay R., Krieg M., Schmidt H. and Voigt K. D. *Acta endocr., Copenh.* **80** (1975) 592–602.

31. Fang S. and Liao S.: *Molec. Pharmac.* **5** (1969) 428–433.
32. Mainwaring W. I. P., Mangan F. R., Fehery P. A. and Freifeld M.: *Molec. cell Endocr.* **1** (1974) 113–128.
33. Liao S., Howell D. K. and Chang T. M.: *Endocrinology* **94** (1974) 1205–1211.
34. Bonne C. and Raynaud J. P.: *Molec. cell. Endocr.* **2** (1974) 59–67.
35. Corvol P., Michaud A., Ménard J., Freifeld M. and Mahoudeau J.: *Endocrinology* **97** (1975) 52–58.
36. Bonne C. and Raynaud J. P.: *J. Pharmac.* **5** (1974) 521–532.
37. Mangan F. R. and Mainwaring W. I. P.: *Steroids* **20** (1972) 331–343.
38. Tan S. Y., Antonipillai I. and Pearson Murphy B.E.: *J. clin. Endocr. Metab.* **39** (1974) 936–941.
39. Massa R. and Martini L.: *Gynec. Invest.* **2** (1971/72) 253–270.
41. Mauvais-Jarvis P., Kuttenn F. and Wright F. *Annls Endocr.* **36** (1975) 55–62.
41. Jenkins J. S. and McCoffery V. M.: *J. Endocr.* **63** (1974) 517–526.
42. Voigt W., Fernandez E. P. and Hsia S. L.: *J. biol. Chem.* **245** (1970) 5594–5599.
43. Patwardhan V. V. and Lanthier A.: *J. steroid Biochem.* **6** (1975) 137–141.
44. Lee D. K. H., Bird C. E. and Clark A. F.: *J. steroid Biochem.* **5** (1974) 609–617.
45. Dubé J. Y., Ngo-Thi N. H. and Tremblay R. R.: *Endocrinology* **97** (1975) 211–214.
46. Hoisaeter P. A.: *Acta endocr., Copenh.* **80** (1975) 188–198.
47. Baulieu E. E., Atger M., Best-Belpomme M., Corvol P., Courvallin J. C.: *Vitams Horm.* **33** (1975) 649–736.
48. Voigt W. and Hsia S. L.: *J. biol. Chem.* **248** (1973) 4280–4285.
49. Hsia S. L. and Voigt W.: *J. invest. Derm.* **62** (1974) 224–227.
50. Shimazaki J., Kato N., Nagai H., Yamanaka H. and Shida K.: *Endocr. jap.* **19** (1972) 97–106.
51. Plasse J. C. and Lisboa B. P.: *Acta endocr., Copenh.* Suppl. **173** (1973) 133.
52. Taurog J. D., Moore R. J. and Wilson J. D.: *Biochemistry* **14** (1975) 810–817.
53. Hartiala J., Nienstedt W. and Hartiala K.: *Steroid Lipid Res.* **3** (1972) 178–184.
54. Hay J. B. and Hodgins M. B.: *J. Endocr.* **59** (1973) 475–486.
55. Mauvais-Jarvis P., Bercovici J. P. and Gauthier F.: *J. clin. Endocr. Metab.* **29** (1969) 417–421.
56. Glock G. E. and McLean P. *Biochem. J.* **55** (1953) 400–408.
57. Widnell C. C. and Tata J. R.: *Biochem. J.* **92** (1964) 313–317.
58. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: *J. biol. Chem.* **193** (1951) 265–275.
59. Wilson J. D. and Gloyna R. E.: *Rec. Prog. Horm. Res.* **26** (1970) 309–336.
60. Horst H.-J., Dennis M., Kaufmann J. and Voigt K. D.: *Acta endocr., Copenh.* **79** (1975) 394–402.
61. Mainwaring W. I. P.: *J. reprod. Fert.* **44** (1975) 377–393.
62. Nozu K. and Tamaoki B. I.: *J. steroid Biochem.* **6** (1975) 57–63.
63. Shain S. A. and Boesel R. W.: *J. steroid Biochem.* **6** (1975) 43–50.
64. Johansson R.: *Acta endocr., Copenh.* **81** (1976) 398–408.
65. Johansson R. and Niemi M.: *Acta endocr., Copenh.* **78** (1975) 766–780.