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SCREENING FOR ANTIANDROGENIC ACTIVITY OF SOME 4,5-CYCLO-A-HOMO-B-NOR-ANDROSTANE DERIVATIVES

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SUMMARY

Five 4α, 5-cyclo-A-homo-B-nor-androstane derivatives were assayed *in vivo* on mice for their antiandrogenic activity and the effect was compared with that of cyproterone acetate. The inhibition of dihydrotestosterone binding to rat prostate cytosol by the compounds was correlated to the *in vivo* effect. The antiandrogenic action of 17β-acetoxy-4α, 5-cyclo-A-homo-B-nor-5α-androst-1-en-3-one was found to be comparable with that of cyproterone acetate. The compound exhibited an antirenotrophic but not a corticoid-like effect. Its teratogenicity assayed on chick embryos was similar to that of spirolactones.

INTRODUCTION

Until now, the synthetic efforts provided in cyproterone acetate [1] and 17α-methyl-B-nortestosterone [2] two most potent steroid antiandrogens which have been widely used not only in experimental endocrinology but have found their place also as a useful therapeutic tool in the clinical treatment of disorders caused by an excess of androgens.

In the search for other potential antiandrogenic steroids we investigated several 4,5-cyclo-A-homo-B-norandrostane derivatives in which the structural features of cyproterone as well as those of 17α-methyl-B-nortestosterone are combined. Their biological activity was tested *in vivo* and compared with their apparent inhibition constants for dihydrotestosterone binding in cytosol of rat prostate.

MATERIALS AND METHODS

Steroids. The compounds we are dealing with have been prepared by Simmons-Smith methylenation of the corresponding unsaturated B-norsteroid derivatives [3] as described in detail elsewhere [4]. Their characteristic physicochemical data are listed in Table 1.

Binding of steroids to rat prostate cytosol. Determination of apparent inhibition constants (K_i) for assayed steroids was carried out as described by Verhoeven *et al.* [5] using [^{3}H]-5α-dihydrotestosterone binding to rat prostate cytosol as outlined here:

Wistar rats were orchidectomized 24 h before killing. The animals were killed by decapitation and exsanguination. Rat prostates were removed, minced and homogenized in 2.5 vol. of Tris–EDTA–glycerol (TEG) buffer (20 mM Tris–HCl, pH 7.4; 1.5 mM EDTA and 10% glycerol). Centrifugation was performed in Spinco ultracentrifuge at 105 000 *g* for 1 h.

Labelling of cytosol receptors was carried out by incubation of the supernatant for 4 h at 0° in the presence of 2×10^{-10} M [1,2 4,5,6,7 (*n*)-^{3}H] 5α-dihydrotestosterone (S.A. 165 Ci/mmol, Radiochemical Centre, Amersham). To evaluate the amount of

Table 1. Characteristic data on 4,5-cyclo-A-homo-B-norandrostanes under study

Compound No.	Name	m.p. °C	Optical rotation $[\alpha]_D^{20}(CHCl_3)$	I.R. cm^{-1}			
I	4α,5-Cyclo-A-homo-B-nor-5α-androstan-3,17-dione	182–183	+35° (c 1.37)				
II	17β-Hydroxy-17α-methyl-4α,5-cyclo-A-homo-B-nor 5α-androstan-3-one	185–186	−84° (c 1.39)	3615	3085	1682	
III	17β-Acetoxy-4α,5-cyclo-A-homo-B-nor-5α-androstan-3-one	151–152	−31° (c 1.42)				
IV	17β-Acetoxy-4α,5-cyclo-A-homo-B-nor-5α-androst-1-en-3-one	112–113	+234° (c 1.44)	1665 1047	1621 1036	1740	1246
V	17β-Hydroxy-17α-methyl-4α,5-cyclo-A-homo-B-nor-5α-androst-1-en-3-one	179–180	+204° (c 1.16)	3615	1620	1653	

steroid bound to nonsaturable binding proteins, parallel incubations were performed in which the unlabelled dihydrotestosterone was added to reach the final concentration of 1.7×10^{-6} M. [^{3}H]5α-dihydrotestosterone and unlabelled competitors (steroids investigated in 8 various concentrations in the range of 2×10^{-13}–2×10^{-10} M) were dissolved in 0.25 ml of TEG buffer and the incubation was started by the addition of 0.75 ml of freshly prepared cytosol.

Precipitation of the receptor-bound androgens was achieved by the addition of an equal vol. of 70% saturated solution of ammonium sulphate at 0° during 15 min. The solution was allowed to stand for 30 min and then centrifuged for 20 min at 10 000 *g*, the supernatant was decanted and the precipitate was resuspended in 0.9 ml of TEG buffer containing 2 mg of bovine albumin. The steroid–protein complex was immediately reprecipitated by the addition of 0.9 ml of 70% saturated ammonium sulphate, allowed to stand for 45 min and centrifuged down as outlined above. After decanting and drying the tube walls with filter paper the final pellet was resuspended in 1 ml of TEG buffer and in an aliquot the radioactivity was measured in Betaszint 500 liquid scintillation spectrometer.

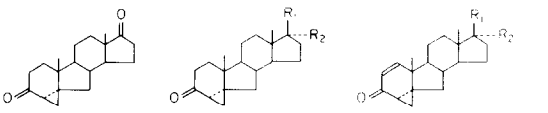
For the displacement curves 8 various concentrations of the competitor were used. K_i-values corresponding to the concentrations of competitors which yield a 50% inhibition of the original amount of radioligand binding were computed from logit-log plot by the means of the method of minimal squares.

Bioassay for antiandrogenic activity. For screening of antiandrogenic activity *in vivo* simultaneous administration of testosterone propionate and of potential antiandrogens to castrated male mice was used. Male strain H mice (Velaz, Praha) weighing approx. 35 g were fed on a standard laboratory diet Velaz containing 23% of proteins with water ad libitum. The animals were kept in an indirectly illuminated room with controlled temperature 24 ± 2°. The mice were castrated 21 days before starting the administration of steroids and kept in groups of 7 animals each.

To the castrates vehiculum or testosterone propionate or testosterone propionate in combination with antiandrogen was administered s.c. daily (0.1 ml) for three weeks. Each steroid treated mouse received in total 2.5 mg of testosterone propionate and those receiving the combination additionally 20 mg of antiandrogen in 20 equal doses. Vehiculum consisted of olive oil and sorbimacrogel 7:1 (v/v).

At the termination of the experiment the animals were killed in ether narcosis, weighed and seminal vesicles, kidneys, adrenals and spleens were removed, cleaned and weighed on a torsion balance. Protein content was determined by the method of Lowry *et al.* [6]. The organ weights were expressed in relative values (mg/100 g b.w.) and the results were evaluated statistically by means of Student's *t*-test.

Teratogenic effect of the compound IV was assayed on chick embryos as described by Jelínek *et al.* [7].



I II R^1 = OH, R^2 = CH_3 III R^1 = OAc, R^2 = H IV R^1 = OAc, R^2 = H V R^1 = OH, R^2 = CH_3

Fig. 1.

Steroid was administered intragerminally or intraamniotically, respectively, at the development stages 9–11, 11–14, 17–20 and 21–25 of Hamburger and Hamilton [8] in 3 μl of dimethylsulfoxide in amounts of 10^{-4}–3×10^{-11} g to 166 embryos and 42 embryos were used as controls. At day 8 of incubation the embryos were examined morphologically.

RESULTS

Displacement of labelled 5α-dihydrotestosterone from its binding to the rat prostate cytosol was used for the determination of apparent inhibition constants of 5 potential antiandrogens. The values found for the new compounds as well as for cyproterone acetate and 17α-methyl-B-nortestosterone are listed in Table 2.

Bioassay was carried out on castrated mice to which testosterone propionate was administered in a dose approx. ten times lower than that of antiandrogens. As reference compound cyproterone acetate was used in similar manner. The body weights, organ relative weights and protein content of kidneys are shown in Table 3. Significance of the difference is calculated in relation to testosterone administration only and to the combination of androgen and cyproterone acetate.

The teratogenicity of the compound IV which promises to be an effective antiandrogen, was tested in chick embryos. No changes in development were found up to the dose of 3×10^{-5} mg. The teratogenic range occurs between 3×10^{-3}–3×10^{-5} mg per embryo. When administered on the 2nd day of incubation in higher concentrations compound IV kills the embryos by destruction of heart development and

Table 2. Values of apparent inhibition constants (K_i) of androgens and antiandrogens, corresponding to the concentration of competitor which yields 50% of inhibition of the original [^{3}H]5α-dihydrotestosterone binding in rat prostate cytosol

Competitor	K_i (nM)
testosterone	1.7
cyproterone acetate	3.7
17α-methyl-B-nortestosterone	0.7
compound I	1162
compound II	>1500
compound III	>1500
compound IV	1.1
compound V	24

Table 3. Body weights, relative organ weights and protein content of kidney of castrated mice treated with testosterone propionate TP (2.5 mg during 3 weeks) and assayed steroid (20 mg during 3 weeks). Mean values ± S.D. of groups of 7 animals each are given

Group	TP	Treatment antiandrogen	Body weight g	Seminal vesicles mg/100 g	Spleen mg/100 g	Adrenals mg/100 g	Kidneys g/100 g	Protein content in kidney %
1	+	—	40.57* ±2.13	487.2* ±99.1	371.7* ±48.8	14.00 ±1.65	2.22* ±0.12	7.80 ±0.82
2	+	Cyproterone acetate	36.78† ±2.20	207.1† ±43.2	179.7† ±27.5	17.63 ±3.26	1.78† ±0.15	8.49 ±1.05
3	+	IV	41.43* ±3.20	233.7* ±26.8	385.2* ±88.2	15.13 ±2.91	1.86† ±0.13	7.90 ±0.65
4	+	V	37.86 ±4.55	354.4*† ±72.3	416.1* ±68.7	19.34 ±5.10	2.08 ±0.25	6.50*† ±0.77
5	+	I	40.71 ±3.53	364.3* ±92.3	466.6†* ±57.7	12.23* ±1.77	2.11* ±0.15	8.20 ±0.89
6	+	II	39.86 ±3.44	369.8* ±70.4	443.8* ±71.1	14.43 ±1.83	2.00 ±0.21	8.91 ±0.94
7	+	III	38.43 ±0.73	380.0* ±88.2	382.2* ±83.6	14.88 ±1.89	2.01†* ±0.13	9.34 ±1.47
8	−	—	36.57* ±2.44	31.5†* ±2.6	398.6* ±52.5	20.26† ±1.76	1.39†* ±0.12	11.39*† ±1.21

* Difference against group 2 (testosterone + cyprotenone acetate) significant at the level $P < 0.05$. † Difference against the group 1 (testosterone) significant at the level $P < 0.05$.

blood circulation. Administration on later development stages causes variable malformations with prevailing schises and upper limb malformations. The teratogenic range of the compound IV is about one order higher than that of spironolactone and by two orders lower than that of selenium.

DISCUSSION

Among five 4,5-cyclo-A-homo-B-norandrostane derivatives tested for their potential antiandrogenicity only 17β-acetoxy-4α,5-cyclo-A-homo-B-nor-5α-androst-1-en-3-one (IV) and to lower degree 17β-hydroxy-17α-methyl-4α,5-cyclo-A-homo-B-nor-5α-androst-1-en-3-one (V) were effective competitors of 5α-dihydrotestosterone binding to the rat prostate receptors. The values obtained for simultaneously tested compounds, previously used in the experiments of Verhoeven *et al.* [5], were in reasonable agreement with the values reported by the authors of the method. Compound IV is a more potent competitor than antiandrogen cyproterone acetate, compound V is comparable in this respect with cyproterone or spironolactone [5], known as active antiandrogens, too.

The inhibition of androgen binding in prostate was also in keeping with the biological effect obtained in *in vivo* experiments. Compounds IV and V were active antiandrogens in the reduction of the seminal vesicles weights. The former compound did not differ significantly from the antiandrogenic activity of cyproterone acetate, the latter was a weaker androgen competitor. Compound I, II and III revealed no potent antiandrogenic activity. Hence, at least one double bond in ring A seems to be essential for the antiandrogenic activity.

The antiandrogen IV is also antirenotrophic similarly as cyproterone acetate, but unlike cyproterone acetate it does not reduce the body weight and relative weight of spleen [8]. Compound V has no influence on the body weight and relative organ weight of spleen and kidney, however, it reduces the protein content in the kidney. The remaining three compounds exert no marked organ effects in doses administered, except for the increase of spleen weight in compound I and II and some effect on the adrenal weight in the former one.

It can be concluded that 17β-acetoxy-4α,5-cyclo-A-homo-B-nor-5α-androst-1-ene-3-one (IV) may be considered as a prospective antihormone with antiandrogenic potency close to that of cyproterone acetate, however lacking its corticoid-like action on spleen and possessing only low antianabolic activity and no gestagenic effects. The compound is only somewhat more teratogenic than spironolactones. For these properties it deserves further investigation.

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