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Endocrine Properties of the Testosterone 5α-Reductase Inhibitor Turosteride (FCE 26073)

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Turosteride was tested in a series of studies for its effect on 5α-reductase and for its possible influence on other steroidogenic enzymes and on steroid receptors. The compound was found to inhibit human and rat prostatic 5α-reductases with IC_{50} values of 55 and 53 nM, respectively, whereas it caused a less marked inhibition of the dog enzyme (IC_{50} 2.2 μM). Turosteride showed no relevant effect on rat adrenal $C_{20,22}$-desmolase (IC_{50} 254 μM) and human placental aromatase ($IC_{50} > 100$ μM), and only at relatively high concentrations it caused inhibition of human placental 5-ene-3β-hydroxysteroid dehydrogenase-isomerase (3β-HSD-I) (IC_{50} 2.5 μM). Turosteride was found to be a selective 5α-reductase inhibitor showing no noteworthy binding to receptors for androgens (relative binding affinity, RBA, 0.004%), estrogens (≤ 0.005%), progesterone (< 0.005%), glucocorticoids (< 0.01%) and mineralocorticoids (< 0.03%). Its biochemical profile was similar to that of finasteride, whereas 4-MA (17β-*N*,*N*-diethyl-carbamoyl-4-methyl-4-aza-5α-androstan-3-one) was confirmed to be a non-selective 5α-reductase inhibitor, showing a degree of binding affinity to the androgen receptor (RBA 0.1%) and a marked inhibition of 3β-HSD-I (IC_{50} 32 nM). When given orally in immature castrated rats together with subcutaneous testosterone propionate (TP) for 7 consecutive days, turosteride reduced the ventral prostate and seminal vesicle growth promoting effect of TP, with IC_{50} values of ≈5 and 6.7 mg/kg/day, whereas levator ani weight was unchanged. In comparison, 4-MA was approx. 3-fold less potent than turosteride in reducing the prostate and seminal vesicle weights and caused a marked reduction of levator ani weight, thus showing its unselectivity.

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INTRODUCTION

The enzyme 4-ene-3-oxosteroid-5α-reductase (5α-reductase) is a NADPH-dependent enzyme that catalyses the conversion of testosterone into the potent tissue-specific androgen 5α-dihydrotestosterone (DHT). This enzymatic activity is localized in some androgen-target tissues, such as the prostate, seminal vesicle, epididymis and skin, where the androgenic stimulus is mediated by DHT, derived from testosterone [1, 2]. On the contrary, in other androgen-sensitive tissues, such as the skeletal muscle and the central structures controlling male sexual drive, the androgenic stimulus is mediated by testosterone itself.

Inhibition of 5α-reductase provides a novel and selective approach to androgen deprivation in DHT-target tissues, without affecting testosterone-target structures. On the contrary, androgen deprivation through androgen receptor antagonists or LHRH analogues affects both DHT- and testosterone-mediated effects. Specific inhibitors of 5α-reductase may be useful in controlling pathological conditions dependent on DHT, such as benign prostatic hyperplasia, acne, female hirsutism and male pattern baldness [3]. A number of steroidal compounds have been described as 5α-reductase inhibitors, including 17β-carboxyl-4-androsten-3-one, 6-methylene-4-pregnen-3,20-dione, 17β-carboxamido-4-aza-androstenes [2] and 3-carboxy-17β-carboxamido-androstenes [3, 4]. Recently one of such compounds, finasteride [MK 906; *N*-(2-methyl-2-propyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide] [2, 5], has been introduced on the market for the treatment of symptomatic benign prostatic hyperplasia [6, 7].

As part of a development program to select a novel 5α-reductase inhibitor, Farmitalia Carlo Erba's laboratories identified the steroidal derivative turosteride [FCE 26073; 1-(4-methyl-3-oxo-4-aza-5α-androstane-17β-carbonyl)-1,3-diisopropylurea] (Fig. 1) [8, 9]. In the present report we describe a series of *in vitro* studies aimed at characterizing the inhibitory effect of turosteride on 5α-reductase of prostatic tissue from different species, its possible influence on other steroidogenic

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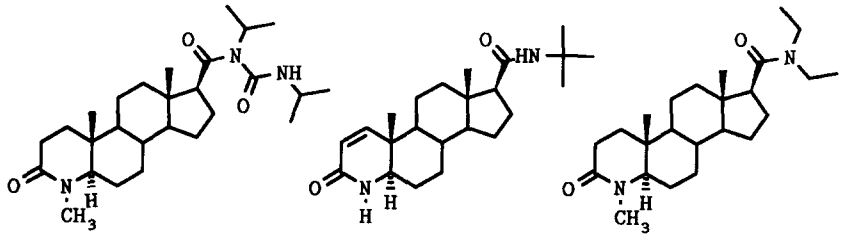

Fig. 1. Chemical structures of turosteride (FCE 26073), finasteride (MK 906) and 4-MA.

enzymes and its affinity for steroid hormone receptors. In addition, the *in vivo* 5α-reductase inhibitory effect of turosteride was studied in castrated rats treated with testosterone propionate (TP). The effects of turosteride were compared to those of the 5α-reductase inhibitors finasteride and 4-MA (17β-*N*,*N*-diethyl-carbamoyl-4-methyl-4-aza-5α-androstan-3-one) [5, 10]. The structures of the above mentioned compounds are reported in Fig. 1.

EXPERIMENTAL

Chemicals

Turosteride, finasteride, 4-MA, 4-hydroxyandrost-4-ene-3,17-dione (formestane) and mifepristone (the progesterone antagonist RU 38486 from Roussel Uclaf) were synthesized at the research laboratories of Farmitalia Carlo Erba (Milano, Italy). Cyproterone acetate, flutamide and tamoxifen citrate were kindly provided by Schering AG, Essex Italia and Farmos. Spironolactone and progesterone where purchased from Aldrich and Roussel Uclaf. The aromatase inhibitor aminoglutethimide was purchased from Farmitalia Carlo Erba. Estradiol valerate (Progynon Depot®) was from Schering AG. [4-^{14}C]Testosterone (50 mCi/mmol), [26-^{14}C]cholesterol (55 mCi/mmol), [1β-^{3}H]androst-4-ene-3,17 dione (28 Ci/mmol), [4-^{14}C]pregnenolone (57 mCi/mmol), [1,2,4,5,6,7-^{3}H]DHT (128 Ci/mmol), [2,4,6,7-^{3}H]17β-estradiol (95 Ci/mmol), [17α-methyl-^{3}H]promegestone (86 Ci/mmol), [6,7-^{3}H]dexamethasone (44 Ci/mmol), [1,2,6,7-^{3}H]aldosterone (82 Ci/mmol), and unlabelled promegestone were purchased from New England Nuclear. All the other unlabelled steroids were purchased from Sigma Chemical Co. NADPH tetrasodium salt and NAD free acid were purchased from Boehringer Mannheim. Aluminium oxide 60G neutral type E (Alumina) and glycine were purchased from Merck. Activated charcoal Norit A and dextran T-70 were purchased from Serva and Pharmacia Fine Chemicals.

Preparation of the solutions

For the *in vitro* tests, stock solutions of the compounds (at 10 mM) were prepared in methanol, and further diluted with methanol and then with the pertinent buffer. The amount of methanol in the final incubation volume was always $\leqslant 3\%$, and a similar amount of solvent was included in the control sample of each assay. For the *in vivo* studies the compounds were suspended in 0.5% methocel (A-4C Premium, Dow Chemical) containing 0.4% Tween 80 (Merck) when given orally, or dissolved in benzylic alcohol and diluted in sesame oil when given subcutaneously.

Source of tissues and animals

Fresh surgical specimen from human benign hyperplastic prostate and fresh human placenta at term were provided by the Hospital of Rho (Milano, Italy). The other required tissues for the *in vitro* tests were removed from the following animal species, supplied by Charles River, Italy: adult male Beagle dogs, adult female (150–200 g) and male (200–300 g) Crl:CD^{R}(SD)BR rats and immature female New Zealand White rabbits (800–900 g). Prepuberal 22-day-old male Crl:CD^{R}(SD)BR rats were used for the *in vivo* studies.

In vitro 5α-reductase inhibition

Inhibition of the conversion of testosterone to the 5α-reduced products DHT and 3α(β),17β-androstanediol was evaluated using the particulate fraction (140,000 *g* pellet) from homogenate of human, rat or dog prostate as the enzyme source, as described by Liang *et al.* [11]. All the assays were performed in a final incubation volume of 0.5 ml. The assay for human 5α-reductase was performed in 40 mM Tris–HCl buffer pH 5.5, containing 1 mM dithiothreitol, 5 mM NADPH and ≈0.3 mg protein of the enzyme preparation. The assay for rat and dog 5α-reductase was carried out in 40 mM phosphate buffer pH 6.5, containing 1 mM dithiothreitol, 0.5–5 mM NADPH and ≈0.6–1 mg protein of the enzyme preparation. For each assay, various concentrations of the azasteroids, in duplicate, were incubated with 1 μM [^{14}C]testosterone for 30 min (60 min for dog enzyme) at 37°C and the reaction was terminated by addition of 2 ml cold diethyl ether containing unlabelled testosterone, DHT, 3α- and 3β,17β-androstanediol (10 μg each). The organic phase was separated by centrifugation, evaporated under N_2 and resuspended in ethyl acetate. Testosterone metabolites were separated in TLC on Silica gel 60 F_{254} plates (Merck) using chloroform–acetone–*n*-hexane (2:1:2, by vol) as the developing solvent system. Radioactivity on the plate was

scanned and analysed from quantitative plots printed by a TLC-analyser (Berthold). The fractional 5α-reduction of testosterone was calculated by relating the ^{14}C-radioactivity in the 5α-reduced metabolite regions (DHT, 3α- and 3β,17β-androstanediols) to the total radioactivity in the testosterone and 5α-reduced metabolite regions.

$C_{20,22}$-desmolase inhibition

Inhibition of $C_{20,22}$-desmolase (cholesterol side-chain cleavage enzyme) was evaluated by measuring the amount of [1-^{14}C]isocaproic acid formed during the conversion of [26-^{14}C]cholesterol to pregnenolone, as described by Rabe *et al.* [12], using mitochondrial preparations from male rat adrenals as the enzyme source. The assay was performed as described previously [13]. Shortly, various concentrations of the tested compounds, in duplicate, were incubated with 10 μM [^{14}C]cholesterol for 20 min at 37°C, in a final volume of 0.5 ml. Unchanged cholesterol was separated from isocaproic acid by alumina adsorption.

Aromatase inhibition

Microsomes were prepared from human placenta according to Ryan [14]. Aromatase activity was tested in the assay of Thompson and Siiteri [15], which determines the rate of aromatization by measuring the tritiated water released from [1β-^{3}H]androstenedione. The assay was carried out in a final volume of 0.5 ml, in 10 mM phosphate buffer pH 7.5, containing 100 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 100 μM NADPH and ≃7 μg of proteins. Various concentrations of the tested compounds, in duplicate, were incubated for 15 min at 37°C with 50 nM [^{3}H]androstenedione and the assay was terminated by the addition of 4 ml cold chloroform. The radioactivity in the water phase was determined by liquid scintillation counting in Rialuma.

5-Ene-3β-hydroxysteroid dehydrogenase-isomerase (3β-HSD-I) inhibition

Inhibition of 3β-HSD-I, the enzyme that catalyses the conversion of 5-ene-3β-hydroxysteroids to 4-ene-3-oxosteroids, was evaluated by measuring the amount of [^{14}C]progesterone formed from [^{14}C]pregnenolone, using microsomal preparations from human placenta as the enzyme source, as described by Ayub and Stitch [16]. The assay was performed in a final volume of 0.5 ml, in 25 mM phosphate buffer pH 7.5, containing 1 mM dithiothreitol, 0.25 M sucrose, 1 mM EDTA, 1 mM NAD, 2 μM [^{14}C]pregnenolone and various concentrations (in duplicate) of the tested compounds. After 5 min pre-incubation at 37°C in a shaking bath, the reaction was started by addition of the enzyme preparation (≈60 μg protein). After 10-min further incubation, the reaction was terminated by addition of 0.5 ml chloroform containing unlabelled pregnenolone and progesterone (50 μg each). After extraction with 2 ml of diethyl ether (twice), the organic phase was separated, evaporated and resuspended in ethanol. An aliquot was applied to silica gel 60 F_{254} plates (Merck) using chloroform–acetone (185:15, v/v) as the developing solvent system. Radioactivity on the plate was scanned by a TLC-analyser (Berthold). 3β-HSD-I activity was calculated by relating the ^{14}C-radioactivity in the progesterone region to the total radioactivity in the progesterone and pregnenolone regions.

Steroid receptor binding affinity

Binding of the compounds to cytoplasmic steroid receptors was determined by standard dextran-coated charcoal (DCC) adsorption techniques [17]. Androgen receptor (AR) was prepared from prostatic tissue of both adrenalectomized and orchidectomized adult rats, to increase specific binding [17]. Animals were castrated 3 days after adrenalectomy and sacrificed the next day. Estrogen receptor (ER) was prepared from uterine tissue of ovariectomized (24 h) adult rats [17]. Progesterone receptor (PR) was prepared from uterine tissue of immature rabbits primed with 20 μg/kg s.c. of estradiol valerate and sacrificed on the fifth day [18]. Glucocorticoid receptor (GR) was prepared from thymus of adrenalectomized (7 days) adult male rats [19]. Mineralocorticoid receptor (MR) was prepared from kidneys of adrenalectomized adult male rats [20]. Seven days after adrenalectomy animals were anesthetized with phenobarbitone (30 mg/kg i.p.), exsanguinated and kidneys were perfused with 0.9% saline via the abdominal aorta.

Cytoplasmic receptors were prepared in 10 mM Tris–HCl buffer pH 7.4, containing 1.5 mM EDTA and 1 mM dithiothreitol (TED buffer). Tissues were homogenized with a Polytron PT-10 in ice-cold TED buffer in a weight–volume ratio of 1:10 for AR, 1:20 for ER, 1:40 for PR, 1:5 for GR and 1:1 for MR preparation. Glycerol (20%) and monothioglycerol (12 mM) were added to TED buffer for PR preparation. The homogenate was centrifuged at 105,000 *g* at 2°C for 1 h and the cytosol was immediately used for competitive steroid binding assay. The assays were performed in 0.4 ml final incubation volume containing an aliquot of the cytosol, 1 nM of the specific ^{3}H-labelled ligand (3 nM for [^{3}H]aldosterone) and various concentrations, in duplicate, of the tested compounds or the specific unlabelled ligand. After 2 h incubation (3.5 h for MR) at 0°C, the bound and free ^{3}H-labelled steroids were separated and bound radioactivity was determined. Non-specific binding was determined in the presence of 100 μM specific unlabelled ligand. The concentration of each compound required to reduce specific ^{3}H-steroid binding by 50% (IC_{50}) was determined and the relative binding affinity (RBA) of each compound was calculated by considering as 100 the potency of the specific ligand.

Protein assay

Protein content in the various preparations was determined using the method of Lowry *et al.* [21] with bovine serum albumin as standard.

Androgenic activity

Prepuberal 22-day-old male rats were castrated via scrotal incision under light ether anesthesia. Starting on the seventh day after orchiectomy, the rats were treated for 7 consecutive days either with the tested compounds (orally) or with TP (0.3 mg/kg/day s.c.). Control animals received the vehicle (0.5 ml/kg p.o. of Methocel/Tween 80). Twenty-four hours after the last dose the rats were sacrificed and ventral prostate and seminal vesicles were removed and weighed.

In vivo inhibition of 5α-reductase

The standard test for the antiandrogenic effect in rats was used. Prepuberal 22-day-old male rats were castrated as described above. Starting on the seventh day after orchiectomy, the rats were treated for 7 consecutive days with a standard s.c. dose of TP (0.3 mg/kg/day) alone or together with various oral (or s.c.) doses of the tested compounds. Twenty-four hours after the last dose the rats were sacrificed and ventral prostate, seminal vesicles and levator ani muscle were removed and weighed. Control animals (TP-treated controls) received the pertinent vehicle (0.5 ml/kg p.o. of Methocel/Tween 80, or 0.2 ml/kg s.c. of sesame oil). In each experiment one group of castrated rats was not treated with TP (castrated controls). The mean percentage of inhibition of the TP-induced hypertrophic response was calculated according to the following formula: % inhibition $= 100 \times (C_{TP} - D)/(C_{TP} - C_C)$, where C_{TP}, C_C and D are the mean organ weight of TP-treated control, castrated control and drug treated group, respectively.

RESULTS

The abilities of turosteride to inhibit the 5α-reduction of testosterone (1 μM) from human, rat and dog prostatic tissues are reported in Table 1. Turosteride was found very effective in inhibiting both the human and the rat enzyme, with IC_{50} values of 55 and 53 nM, whereas it was weaker as an inhibitor of the dog enzyme (IC_{50} 2.2 μM). The reference compound finasteride was slightly more potent than turosteride in inhibiting human (IC_{50} 31 nM) and rat (IC_{50} 33 nM) enzymes, but slightly less effective than turosteride in inhibiting dog 5α-reductase (IC_{50} 3.2 μM). 4-MA was found to be as potent as finasteride in inhibiting human (IC_{50} 28 nM) and rat (IC_{50} 37 nM) enzymes.

Table 1. In vitro inhibition of human, rat and dog prostatic 5α-reductase

	IC_{50} (nM)		
Compound	Human	Rat	Dog
Turosteride	55 (4)[a]	53 (4)	2200 (2)
Finasteride	31 (5)	33 (4)	3200 (2)
4-MA	28 (3)	37 (3)	—

The compounds were incubated for 30 min with [^{14}C]testosterone (1 μM).
[a]Number of assays.

Table 2. Inhibition of rat adrenal desmolase[a] and human placental aromatase and 3β-HSD-I[b]

	IC_{50} (μM)		
Compound	Desmolase[c]	Aromatase[d]	3β-HSD-I[e]
Turosteride	254[f]	>100	2.5
Finasteride	242	>100	>3.0
4-MA	>300	>100	0.032
Aminoglutethimide	33	3.1	—
Formestane	—	0.043	—

[a]Cholesterol side-chain cleavage enzyme. [b]5-Ene-3β-hydroxysteroid dehydrogenase-isomerase. [c,d,e]Substrate: [^{14}C]cholesterol (10 μM), [1β-^{3}H] androstenedione (50 nM) and [^{14}C]pregnenolone (2 μM). [f]Results are the average of two separate assays.

The possible inhibition of turosteride on rat adrenal desmolase was estimated from the decrease in the formation of pregnenolone from cholesterol (10 μM), using aminoglutethimide as reference standard. Aminoglutethimide caused inhibition of desmolase with an IC_{50} of 33 μM (Table 2). Adrenal desmolase was affected by both turosteride and finasteride only at very high concentrations (IC_{50} 254 and 242 μM, respectively), approximately four orders of magnitude higher than the concentrations inhibiting rat prostatic 5α-reductase. The inhibitory effect on desmolase by 4-MA was very weak, only 25% inhibition being observed at the highest tested concentration of 300 μM.

The possible inhibitory effect of turosteride on human placental aromatase was studied in comparison to the aromatase inhibitors formestane and aminoglutethimide. These reference standards caused a marked inhibition of the enzyme, with IC_{50} values of 43 nM and 3.1 μM, respectively (Table 2). Turosteride, like finasteride and 4-MA, caused no modification in aromatase activity even at the very high concentration of 100 μM.

The effect of turosteride on human placental 3β-HSD-I was tested from the decrease in the formation of progesterone from pregnenolone. 4-MA, already described as an inhibitor of this enzyme, was used as reference standard [22]. 4-MA was confirmed to cause a very marked inhibition of 3β-HSD-I (Table 2). In fact, its potency in inhibiting this enzyme activity (IC_{50} 32 nM) was found to be similar to the potency on human prostatic 5α-reductase (IC_{50} 28 nM). Turosteride caused inhibition of 3β-HSD-I activity only at concentrations (IC_{50} 2.5 μM) approx. 80-fold higher than those of 4-MA. Finasteride, at the highest tested concentration of 3 μM, caused only 14% inhibition of 3β-HSD-I.

The possible ability of turosteride to bind to the steroid cytosolic receptors was compared to that of various reference receptor antagonists. Table 3 summarizes the RBA of each compound, by considering as 100% the RBA of the specific unlabelled ligand (DHT, estradiol, progesterone, dexamethasone or aldosterone). The various reference standards showed the expected binding affinities. Turosteride showed no relevant binding affinity to the AR (RBA 0.004%), ER

Table 3. Steroid receptor binding affinity

Compound	Relative binding affinity[a] (% RBA)				
	AR	ER	PR	GR	MR
Turosteride	0.004[b]	≤0.005	<0.005	<0.01	<0.03
Finasteride	0.004	≤0.003	<0.005	<0.01	<0.03
4-MA	0.1	≤0.002	<0.005	<0.01	<0.03
Cyproterone acetate	21	—	—	1	—
Flutamide	0.12	—	—	<0.01	—
Tamoxifen	—	1.5	—	—	—
Mifepristone	—	—	27	112	—
Spironolactone	—	—	—	—	23

[a]Calculated by considering the potency of each specific ligand (DHT, estradiol, progesterone, dexamethasone and aldosterone) as 100%. [b]Results are the average of two separate assays.

(≤0.005%), PR (<0.005%), GR (<0.01%) and MR (<0.03%). Finasteride and 4-MA had a receptor binding profile similar to that of turosteride, with the only exception of a slight binding of 4-MA to the AR (RBA 0.1%), which was 25-fold higher than the binding of both turosteride and finasteride (0.004%).

The possible *in vivo* androgenic activity of turosteride was studied in immature castrated rats. The compound, at the oral doses of 30 and 100 mg/kg/day for 7 days, was found ineffective in increasing ventral prostate and seminal vesicle weights, like finasteride (Fig. 2).

In order to test the inhibitory effect of turosteride on 5α-reductase in an animal model *in vivo*, immature castrated rats were treated daily with s.c. TP, alone or concurrently with the azasteroid, for 7 consecutive days. In a first experiment the effect of oral turosteride was compared to that of finasteride (Fig. 3). Turosteride reduced the hypertrophic response of TP on ventral prostate by 23, 45 and 60% at 1, 3 and 10 mg/kg, showing an ED_{50} of approx. 5 mg/kg. Finasteride inhibited prostate growth by 34, 43, 42 and 59% at 0.3, 1, 3 and 10 mg/kg, showing an ED_{50} of approx. 5 mg/kg, like turosteride. However, since the dose–response curve of finasteride was flatter than that

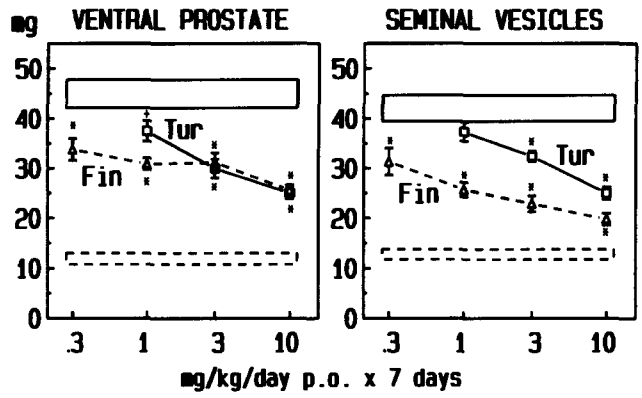

Fig. 3. Effects of oral administration of turosteride and finasteride on TP (0.3 mg/kg/day s.c.)-induced growth of ventral prostate and seminal vesicles in immature castrated rats. Mean ± SE of 7–15 animals/group. In this and succeeding figures the solid and the dashed boxes represent the organ weight in the TP-treated controls and in the castration only controls, respectively. $^{+}P < 0.05$; $^{*}P < 0.01$ vs TP-treated controls (Dunnett's test).

of turosteride, in the low dose-range finasteride was more effective than turosteride. The TP-induced seminal vesicle growth was also reduced by treatment with either compound. However, interestingly, the effect of turosteride was less marked than that of finasteride (ED_{50} 6.7 and 0.8 mg/kg/day, respectively). Neither turosteride nor finasteride caused any change in TP-induced growth of the levator ani muscle, at any tested dose (data not shown).

In further experiments the inhibitory activity of oral turosteride was compared to that of 4-MA (Fig. 4). No effect on prostate and seminal vesicle weights was observed with 4-MA up to 10 mg/kg/day, whereas the compound was effective on both organs at the doses of 30 (52 and 53% inhibition, respectively) and 100 mg/kg/day (80 and 68% inhibition). Turosteride confirmed the previous finding, significantly reducing the TP effect on the prostate and seminal vesicles starting from 1 or 3 mg/kg/day, respectively. In ad-

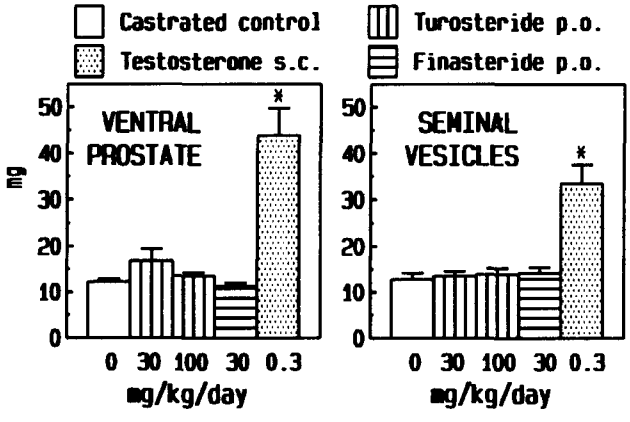

Fig. 2. Effects of 7-day administration of turosteride (p.o.), finasteride (p.o.) or TP (s.c.) on ventral prostate and seminal vesicle weights in immature castrated rats. Mean ± SE of 6–7 animals/group. $^{*}P < 0.01$ vs control group (Dunnett's test).

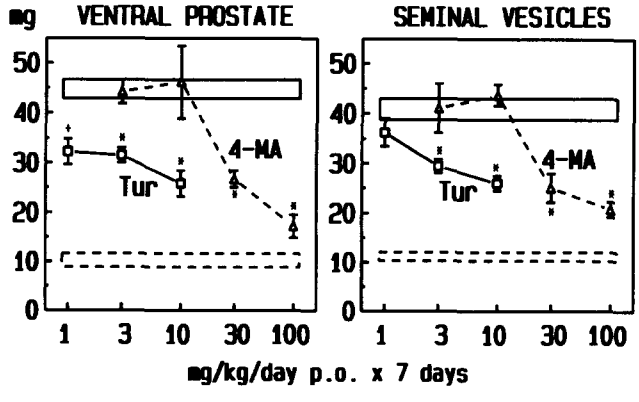

Fig. 4. Effects of oral administration of turosteride and 4-MA on TP (0.3 mg/kg/day s.c.)-induced growth of ventral prostate and seminal vesicles in immature castrated rats. Mean ± SE of 6–14 animals/group.

dition, turosteride dosing, similarly to the previous experiment, caused no change in TP-induced levator ani muscle growth, whereas 4-MA significantly reduced levator ani weight at the doses of 30 and 100 mg/kg/day (data not shown).

The comparison of the effects of oral turosteride to those of the AR antagonist flutamide is reported in Fig. 5. At 10 mg/kg/day turosteride reduced TP-induced prostate and seminal vesicle growth by 67 and 53%, respectively. Flutamide, at the same dose, was more effective, reducing ventral prostate and seminal vesicle growth by 90 and 95%. In addition, levator ani muscle growth was completely suppressed with flutamide, whereas no effect was evident with turosteride (data not shown).

Finally, in a further experiment the effects of turosteride by the s.c. and the oral routes were compared (Fig. 6). At the oral dose of 1 and 10 mg/kg/day, turosteride reduced TP stimulated ventral prostate growth by 26 and 59% and seminal vesicles by 22 and 45%, respectively. Subcutaneous dosing with turosteride was approx. 3-fold less potent than oral treatment in reducing prostate growth, since inhibitions of 32, 36 and 66% were obtained at 3, 10 and 30 mg/kg/day, respectively. At these s.c. doses turosteride reduced the seminal vesicle growth by 24, 47 and 65%, thus becoming as potent as after oral dosing on this target organ.

DISCUSSION

Turosteride has been characterized as a potent and specific inhibitor of testosterone 5α-reductase. As already described for other 4-azasteroid derivatives [11], turosteride was found to be more effective in inhibiting human and rat prostatic 5α-reductases (IC_{50} values of 55 and 53 nM) than the dog enzyme (IC_{50} 2.2 μM). As a comparison, finasteride resulted slightly more potent than turosteride on human and rat enzymes, and slightly less potent on dog enzyme.

The results reported here on the inhibitory effects of finasteride on crude (particulate fraction) enzyme

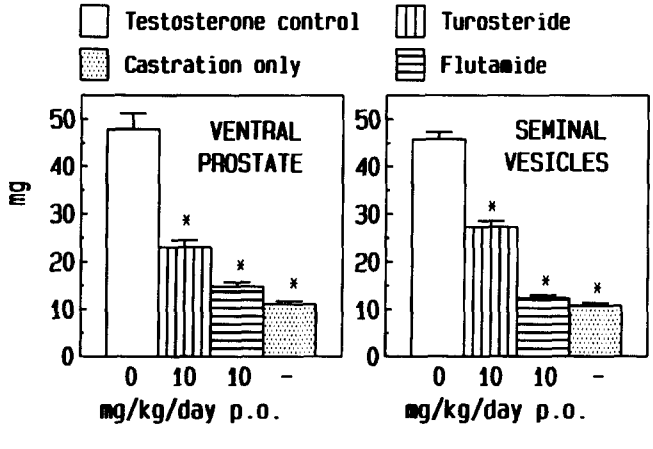

Fig. 5. Effects of 7-day oral administration of 10 mg/kg/day of turosteride or flutamide on TP (0.3 mg/kg/day s.c.)-induced growth of ventral prostate and seminal vesicles in immature castrated rats. Mean ± SE of 11–12 animals/group.

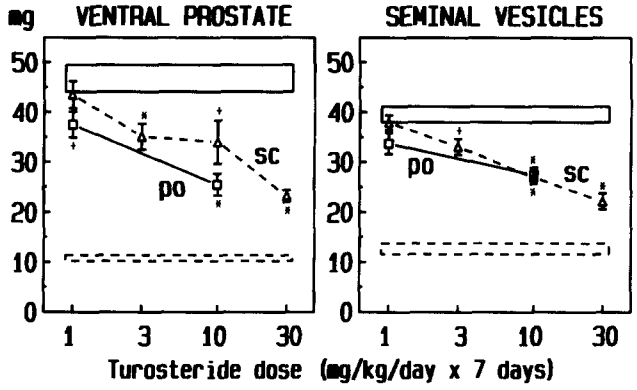

Fig. 6. Effects of the subcutaneous or oral administration of turosteride on TP (0.3 mg/kg/day s.c.)-induced growth of ventral prostate and seminal vesicles in immature castrated rats. Mean ± SE of 7–15 animals/group.

preparations from prostate of different species are in agreement with published data [11]. Recently, two 5α-reductase isozymes, types 1 and 2, have been described either in human [23–26] and rat prostate [23, 26, 27], with pH optima neutral to basic (basic form) and of 5.0–5.5 (acidic form), respectively. The two types of cloned and expressed 5α-reductases were found to have a different chemical structure, and differences were also found in the structures of the same type of isozyme in the two species. As a consequence, recent studies have shown that the two types of rat and human 5α-reductases have different degrees of sensitivity to various inhibitors [23, 27, 28]. As an example, finasteride was found to inhibit the various isozymes in the following decreasing order of potency: rat type 2 (K_i 0.46 nM) > human type 2 (≃5 nM) ≃ rat type 1 (5.8–12 nM) > human type 1 (230–330 nM), thus becoming more selective for type 2 isozymes in both species [24, 26, 27]. Since the crude 5α-reductase enzyme preparations from human and rat prostate have different pH optima, i.e. 5.0–5.5 and 6.5–7.0 [11], it is likely that at these pH conditions (as in the present study) the observed inhibitory effects of tested compounds represent inhibition of the human type 2 and rat type 1 isozyme, respectively. Studies are in progress to characterize the effect of turosteride on human isozymes. No information is available on the existence of dog 5α-reductase isozymes. In the present study, using a crude enzyme preparation from dog prostate (pH optimum of 6.0–6.5), both finasteride and turosteride were found to have a very low potency. However, the relevance of these *in vitro* data is unknown as, e.g. finasteride was found *in vivo* to cause a marked reduction of dog prostate weight [29].

Turosteride, like finasteride, did not cause any noteworthy inhibitory effect on human placental aromatase and rat adrenal desmolase. Turosteride, only at a concentration approx. 50-fold higher than that required for inhibition of human and rat 5α-reductases, was found to inhibit human placental 3β-HSD-I. Finasteride was ineffective on 3β-HSD-I, in agreement with published observations [30], whereas 4-MA was

confirmed to be a potent inhibitor of this enzyme, as effective as in the inhibition of human 5α-reductase, thus resulting in a non-selective 5α-reductase inhibitor [22, 30].

Turosteride, like finasteride, showed no noteworthy binding to the various steroid receptors. The no binding of finasteride to the rat AR has been described previously [11]. A slight binding affinity to the AR was found for 4-MA, in agreement with published data [11].

The *in vivo* results in castrated rats reported here indicate that oral turosteride inhibited the TP-induced hypertrophic response on the ventral prostate and seminal vesicles. In the same experimental conditions finasteride reduced the prostate weight, consistent with previously published results [5], but its potency could not be compared to that of turosteride, since the dose–response curves were not parallel. In fact, in the low dose-range ($\leqslant 1$ mg/kg) finasteride was more effective than turosteride, whereas at the doses of 3 and 10 mg/kg the two compounds were similarly effective. In contrast to turosteride, finasteride was found to reduce seminal vesicle growth at doses lower than those reducing prostate growth, a finding indicating a different drug distribution pattern of the two compounds and/or the existence of different forms of 5α-reductase in these organs. The higher efficacy of turosteride in decreasing rat prostate weight when administered orally than subcutaneously is an unusual finding for a steroidal compound. These results could be due to low adsorption at the injection site and/or more likely to the formation of active metabolite(s) after oral dosing. Neither turosteride nor finasteride influenced the weight of the levator ani muscle up to the maximum tested dose of 10 mg/kg/day p.o., which produced a marked inhibitory effect on prostate growth (59–60% decrease). By contrast 4-MA, although at least 3 times less potent than turosteride in reducing ventral prostate and seminal vesicle growth, caused a marked reduction of TP-stimulated levator ani muscle weight at 30 mg/kg/day, a dose which produced a 52% decrease in ventral prostate growth. This antianabolic effect of 4-MA can be related to an antagonism at the AR level, as shown by its slight binding affinity for the AR [11]. As expected, the AR antagonist flutamide, at the oral dose of 10 mg/kg/day, was found to be highly effective in reducing TP-induced prostate and seminal vesicle growth, as well as the levator ani muscle growth. Since muscular hypertrophy is known to be dependent on the anabolic effect of unchanged testosterone, on account of the absence of 5α-reductase activity in the striatal muscle [31], the ineffectiveness of turosteride, as well as of finasteride, on levator ani muscle weight indicates that the compounds do not antagonize the binding of testosterone to the AR.

In conclusion the *in vitro* results reported here show that turosteride is a potent and selective inhibitor of 5α-reductase. Its antiandrogenic effects observed *in vivo* on rat ventral prostate and seminal vesicles are more likely to be due to 5α-reductase inhibition than to antagonism at the AR level. In fact, turosteride has been demonstrated to have no binding affinity for the AR and no partial androgenic effect *in vivo* in the rat. Its biochemical profile is similar to that of finasteride, whereas 4-MA was confirmed to be a non-selective 5α-reductase inhibitor.

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