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Effects of 5α-Reductase Inhibitors on Intraprostatic Androgens in the Rat

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FCE 27837 is a novel inhibitor of 5α-reductase, the enzyme responsible for the conversion of testosterone (T) to 5α-dihydrotestosterone (DHT). The compound caused inhibition of human and rat prostatic enzymes, with IC_{50} values of 51 and 60 nM, respectively. The *in vivo* effect of FCE 27837 on 5α-reductase was evaluated in adult male rats, treated orally at 10 mg/kg/day for 10 days. The compound caused 33 and 42% reductions in ventral prostate and seminal vesicle weights, respectively. The prostatic content of DHT, measured 6 h after the 10th dose of FCE 27837, was reduced by 75%, whereas T content increased by 442%. Similar effects were observed with 10 mg/kg/day of finasteride, whereas epristeride, tested at the same oral dose, was found to be the least effective compound, decreasing prostate weight by 22% and DHT content by 46%. Castration caused >90% reductions in prostatic weight and prostatic DHT.

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INTRODUCTION

The steroid 5α-reductase enzyme catalyzes the irreversible conversion of testosterone (T) to the potent tissue-specific androgen 5α-dihydrotestosterone (DHT). Inhibition of 5α-reductase provides a novel and selective approach to androgen deprivation in DHT-target tissues, without affecting T-target structures. Finasteride (MK 906) [1], the prototype of 5α-reductase inhibitors, has recently been introduced into the market for the therapy of symptomatic BPH [2]. Other steroidal 5α-reductase inhibitors are undergoing clinical evaluation, including epristeride (SK&F 105657) [3], turosteride (FCE 26073) [4] and MK 963 [5]. Recently, we reported the 5α-reductase inhibitory properties of various 4-azasteroids with fluoro-substituted 17β-amidic side chains [6]. In the present study we describe further endocrinological properties of one such fluoro derivative, FCE 27837 (*N*-[1,1,1-trifluoro-2-oxobut-3-yl]-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide), in comparison with those of finasteride and epristeride. Their *in vivo* effects on 5α-reductase have been studied by measuring the prostatic T and DHT levels after repeated oral treatment in adult male rats.

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MATERIALS AND METHODS

Chemicals

FCE 27837, finasteride and epristeride were synthesized at the research laboratories of Pharmacia/Farmitalia Carlo Erba (Italy). [4-^{14}C]T (50 mCi/mmol), was purchased from New England Nuclear. All the other unlabelled steroids and the protease inhibitors aprotinin and leupeptin were obtained from Sigma Chemical Co., St Louis, MO. Celite ready-to-use for steroid chromatography (Chromatolithe A®) was supplied by bioMérieux.

Preparation of the solutions

For the *in vitro* tests, stock solutions of the compounds were prepared in methanol and diluted with the assay buffer. The amount of methanol in the final incubation volume was always ⩽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).

Animals

Adult male Crl:CD®(SD)BR rats, weighing approx. 250 g at the beginning of the experiment, were supplied by Charles River, Italy. Animals were fed a

commercially available chow (Altromin MT, supplied by Rieper) and water was available *ad libitum*.

In vitro inhibition of 5α-reductase

Inhibition of the conversion of T to the 5α-reduced products DHT and 3α(β),17β-androstanediol was evaluated using the rat prostate and the human hyperplastic prostate as the enzyme sources. The particulate fraction (140,000 ***g*** pellet) from homogenate of adult rat prostates was obtained as described by Liang *et al.* [7]. The assay was performed in a final incubation volume of 0.5 ml, in 40 mM phosphate buffer pH 6.5, containing 1 mM dithiothreitol, 0.5 mM NADPH and ≈700 μg of protein. The human prostatic tissue was homogenized in a w/v ratio of 1:3 with a 100 mM Tris–HCl buffer, pH 7.0, containing 20% glycerol, 100 mM sodium citrate, 100 mM KCl, 1 mM EDTA and 5 mM dithiothreitol (buffer *a*). To protect the enzyme during the preparation process, 0.5 mM NADPH, 1 μM T and 10 μg/ml of the protease inhibitors aprotinin and leupeptin were also added to buffer *a* [8]. The microsomes were then isolated, the pellet resuspended in buffer *a*, also containing the protease inhibitors, and stored at −80°C. Incubations were performed in a final incubation volume of 0.5 ml of buffer *a*, containing 0.5 mM NADPH and ≈300 μg of microsomal proteins. For both enzyme preparations, various concentrations of the inhibitors, in duplicate, were incubated with 1 μM [^{14}C]T for 30 min at 37°C. The samples were then processed as described previously [4].

In vivo effect in adult male rats

Groups of 13–14 male rats were treated orally with FCE 27837, finasteride or epristeride, at a dose of 10 mg/kg/day, once daily for 10 consecutive days. Rats treated with 0.5% Methocel containing 0.4% Tween 80 (2 ml/kg) served as intact controls. Half the animals of each group were sacrificed by decapitation 6 h after the 10th administration, and the remaining animals at 24 h. A group of 6 rats was castrated on the first experimental day and sacrificed 24 h after the last vehicle administration. The following organs were removed and weighed: ventral prostate, seminal vesicles, testes and epididymides. For each group the organ weights of the animals sacrificed at the two experimental times were pooled. The prostates were stored at −20°C for T and DHT determinations.

T and DHT determinations

Prostatic concentrations of T and DHT were measured by specific radioimmunoassays (RIA), after sample extraction and purification on celite columns. Each prostate sample was thawed and homogenized in 4 ml of acetone: acetonitrile mixture (1:1) with a Polytron apparatus. Prostates from two castrated rats were pooled in order to have sufficient tissue for hormone evaluation. After extraction and centrifugation, the organic phase was dessicated, the dried extract was dissolved in 5% methanol aqueous solution and purified on C-18 Amprep minicolumns (Amersham, U.K.), using ethylacetate as the eluting solvent. The dried extract was then dissolved in 0.5 ml isooctane and applied to a celite column (1.2 g of Chromatolithe A® in a 5 ml glass pipette). Elution was then performed with increasing concentrations of ethylacetate in isooctane (0, 6 and 22%). The fractions containing DHT (6% ethylacetate) and T (22% ethylacetate) were evaporated under N_2 and the dried samples were processed for RIA. T and DHT levels in the resuspended samples were estimated, in duplicate, by using the [^{3}H]T and [^{3}H]DHT RIA Kits supplied by bioMérieux and ICN Biomedicals, respectively. The final sensitivity for T and DHT assays were 0.3 and 1 ng per g of prostate, respectively (for a sample of at least 0.2 g).

RESULTS

The abilities of FCE 27837, finasteride and epristeride to inhibit the 5α-reduction of T (1 μM) from human and rat prostatic tissues are reported in Table 1. FCE 27837 was shown to be as potent as finasteride in inhibiting the human enzyme, whereas epristeride resulted as being the most effective compound (IC_{50} values of 51, 51 and 16 nM, respectively). In the rat enzyme, FCE 27837 was slightly less potent than finasteride, like epristeride (IC_{50} values of 60, 32 and 58 nM, respectively).

In order to test the inhibitory effect of the compounds on 5α-reductase in an animal model *in vivo*, adult male rats were treated for 10 days, the DHT target organs were removed and weighed and the T and DHT prostatic contents were measured. Since in a preliminary study in rats we had found that a single oral dose of finasteride (1 mg/kg) and epristeride (3 mg/kg) reduced prostatic DHT at 4–8 h but not 24 h after treatment [9], in the present study we have given the tested compounds at a dose of 10 mg/kg/day and measured the DHT and T prostatic contents both 6 and 24 h after the 10th dose. The 10 day treatment with FCE 27837, finasteride and epristeride did not cause any change in final body weight and body weight gain (data not shown). FCE 27837 caused 33 and 42%

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

Compound	IC_{50} (nM)	
	Human	Rat
FCE 27837	51 (3)	60 (3)
Finasteride	51 (6)	32 (5)
Epristeride	16 (3)	58 (3)

Number of assays in parentheses.
Incubations were performed in the presence of 1 μM [^{14}C]T.

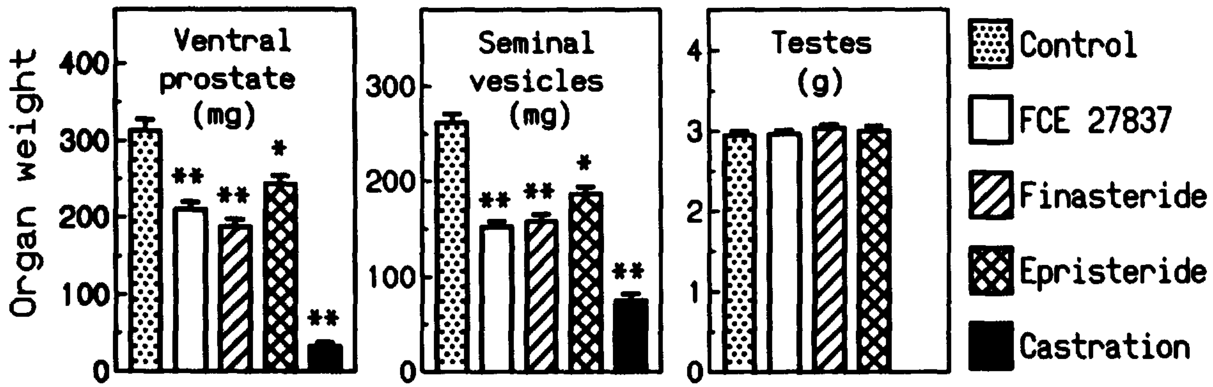

Fig. 1. Effect of repeated oral administration (10 mg/kg/day for 10 days) of FCE 27837, finasteride and epristeride on organ weights in adult male rats. Mean ± SE of 13–14 animals per group. $*P < 0.05$; $**P < 0.01$ vs control group (Dunnett's test).

reduction in ventral prostate and seminal vesicle weights, comparable to the reductions induced by finasteride (40% reduction of both organ weights) (Fig. 1). Epristeride was the least effective compound, causing 22 and 29% reduction of prostate and seminal vesicle weights. As a comparison, castration noticeably decreased the weight of the prostate (90%) and seminal vesicles (72%). FCE 27837, finasteride and epristeride slightly reduced epididymis weight (11, 7 and 6%, respectively; data not shown), whereas no change was observed in testis weight (Fig. 1).

The intraprostatic DHT and T values, measured 6 and 24 h after the last dose, are reported in Fig. 2. Since all the compounds, as well as castration, reduced prostate weight, the results have been expressed in terms of total content of prostatic androgens (ng per

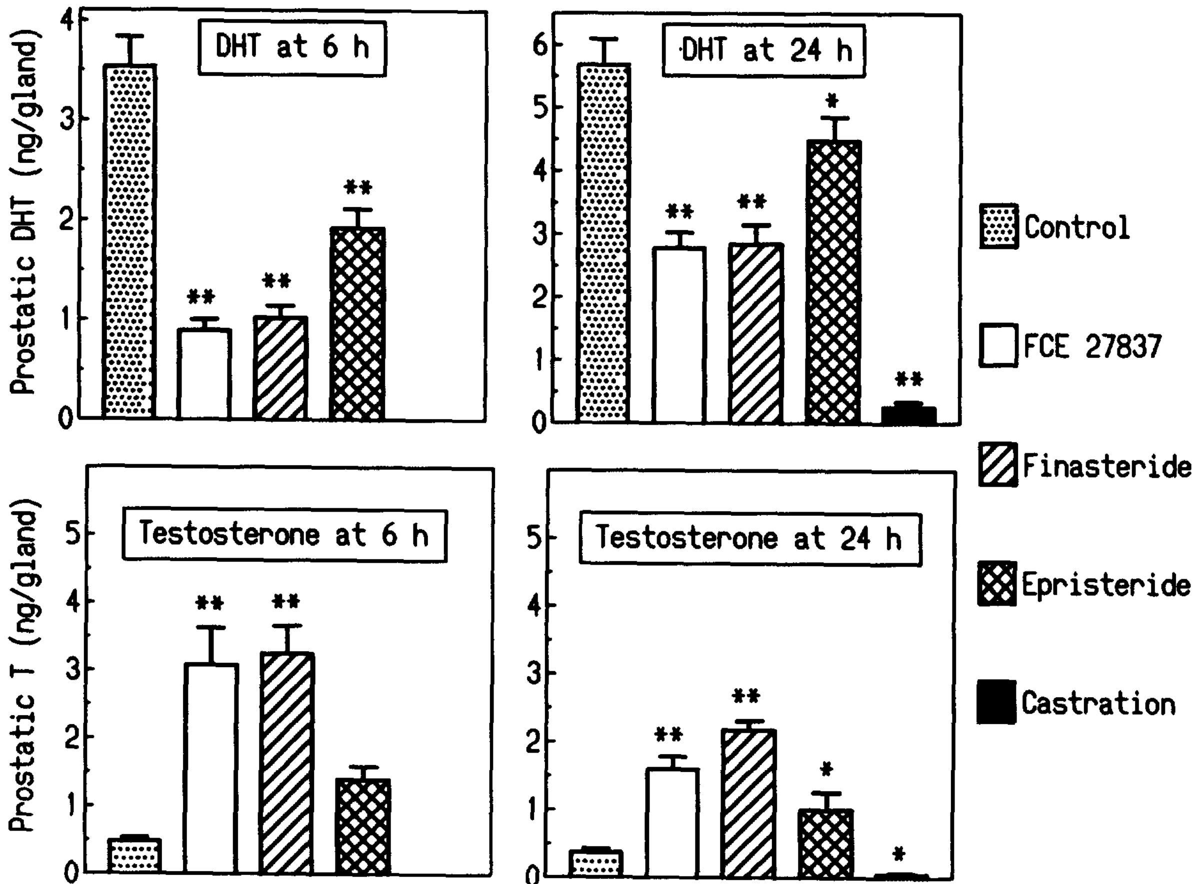

Fig. 2. Effect of repeated oral administration (10 mg/kg/day for 10 days) of FCE 27837, finasteride and epristeride on prostatic content of dihydrotestosterone (DHT) and testosterone (T) in adult male rats. The animals were sacrificed 6 or 24 h after the 10th inhibitor dose. Mean ± SE of 6–7 animals per group (three pools of 2 prostates each for castrated group). $*P < 0.05$; $**P < 0.01$ vs control group (Dunnett's test).

gland). The prostatic DHT and T contents of control rats sacrificed at 6 h were found to be 3.53 and 0.48 ng/gland, respectively, and the average ratio of tissue DHT/T was 8.0. Six hours after the last dose of FCE 27837, finasteride and epristeride, the DHT content was reduced by 75, 71 and 46%, respectively. Such an effect was associated with an increase in prostatic T content by 442, 577 and 90%, respectively. As a consequence, the average DHT/T ratio noticeably decreased from 8.0 in control animals to 0.3 for both FCE 27837 and finasteride, and to only 1.7 for epristeride. At the 24 h sacrifice, the DHT and T contents in control rats were 5.68 and 0.37 ng/gland, respectively, and the average DHT/T ratio was 16.9. At this observation time FCE 27837, finasteride and epristeride caused a reduction in DHT content by 51, 50 and 21% and an increase in T content by 237, 393 and 73%, respectively. Therefore, a decrease in the average DHT/T ratio, although less marked than that observed at 6 h, was still evident, the values being 1.8, 1.3 and 6.2 for FCE 27837, finasteride and epristeride, respectively. In contrast to 5α-reductase inhibitors, castration reduced both DHT (95%) and T (88%) content.

DISCUSSION

Species differences in sensitivity to the inhibitory effect of 5α-reductase inhibitors on the rat and human prostatic enzymes have been found with a series of steroidal compounds [7]. The *in vitro* results reported here show that FCE 27837 is approximately equipotent in inhibiting the rat and the human enzyme, thus suggesting that the *in vivo* studies on rat 5α-reductase may be important in predicting the efficacy on the human prostate. The *in vitro* results on human and rat enzymes reported here with finasteride and epristeride are in agreement with published data [7, 10]. Recently, two 5α-reductase isozymes, type 1 and 2, with different pH sensitivity, have been described in human and in rat prostate [11, 12]. Both finasteride and epristeride have been described as selective inhibitors of human type 2 isozyme [13, 14]. The results described here on the effect of FCE 27837 on the crude enzyme preparation from human prostate (pH optimum at 5.5) likely represent inhibition of the type 2 human isozyme. Studies are in progress to characterize the effect of FCE 27837 on human isozymes.

In a previous study in prepubertal castrated rats supplemented with testosterone, we have shown that FCE 27837, given orally for 7 days, is very effective in reducing prostate growth (61% reduction at 10 mg/kg/day) [6]. We now report that FCE 27837, given orally at 10 mg/kg/day for 10 days in adult rats, is able to reduce prostate and seminal vesicle weight by 33 and 42%, without any effect on testis weight. Similar changes were observed with the same dose of finasteride, whereas epristeride was the least effective compound (22 and 29% inhibition of prostate and seminal vesicle weights). The lower efficacy on rat prostate weight by epristeride in comparison to finasteride confirms published data [15].

In a preliminary study in rats we have found that a single oral dose of finasteride and epristeride reduced prostatic DHT at 4–8 h but not at 24 h [9]. Therefore in this 10 day repeated dose study we have measured DHT content 6 and 24 h after the last dose. To our knowledge no data are available on the 24 h time-course of prostatic DHT suppression after 5α-reductase inhibitor repeated administration, either in rats or humans, the effect being measured at one single time, generally shortly after the last inhibitor dose [15–18]. In this study in rats we have shown that, even at a relatively high dose of both FCE 27837 and finasteride (10 mg/kg/day) given for 10 days, DHT suppression is more marked when measured at 6 h (inhibition of 75 and 71%, respectively) than at 24 h (51 and 50% inhibition). The DHT suppression of both compounds has been found to be associated to a noticeable increase in T content, thus demonstrating the *in vivo* inhibition of 5α-reductase. The DHT suppression caused by 10 mg/kg/day of epristeride was found to be markedly lower (inhibition of 46 and 21% at 6 and 24 h, respectively), in agreement with the lower reduction in prostate weight. Epristeride has also been reported to be several times less effective than finasteride in inhibiting prostatic DHT in humans [17, 18], in spite of its higher *in vitro* potency in inhibiting the human enzyme. Less favourable pharmacokinetic properties of epristeride could likely be the cause for these results. Castration was confirmed to cause $\approx$90% reduction in prostatic DHT and T content and in prostate weight. The three inhibitors of 5α-reductase reported in this study, given at an oral dose of 10 mg/kg/day, have been found to cause less marked reduction (range 22–40%) in prostate weight than did castration. These findings can be ascribed to either a less marked maximal reduction of prostatic DHT content (range 46–75%, 6 h after the last dose) or to a less sustained DHT inhibition (range 21–51%, assayed 24 h after the last dose) resulting from 5α-reductase inhibitor treatment in comparison to castration. In addition, a possible role for increased prostatic T in maintaining prostate weight cannot be ruled out.

The results reported here indicate that FCE 27837 is a potent *in vitro* inhibitor of both human and rat prostatic 5α-reductase. In addition, the compound was found to be a potent *in vivo* inhibitor of the enzyme in rats. Further studies are in progress to characterize the endocrinological properties of this novel 5α-reductase inhibitor.

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