EFFECT OF 17α-METHYL-β-NORTESTOSTERONE
(SK & F 7690) ON THE BINDING IN VITRO
OF 5α-DIHYDROTESTOSTERONE TO MACROMOLECULAR
COMPONENTS FROM THE RAT VENTRAL PROSTATE

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ABSTRACT

Slices from prostate glands of castrated male rats were incubated with
[3H] 5α-dihydrotestosterone in Eagle's tissue culture medium. The labelled
androgen was associated with androphilic macromolecules both in the
prostatic cytosol and the nuclei.

The addition of the anti-androgenic compound, 17α-methyl-β-nortesto-
sterone (SK & F 7690) to the incubation medium inhibited the formation
of the nuclear 5α-dihydrotestosterone-protein complex, and markedly
reduced the cytosol 5α-dihydrotestosterone-protein complex. Likewise, the
uptake of [3H] 5α-dihydrotestosterone by the prostatic nuclear fraction
was reduced by about 40%.

Recent investigations indicate that 5α-dihydrotestosterone (5α-androstan-17β-
ol-3-one) is of primary importance in the mediation of the androgenic message
to susceptible cells. Testosterone is rapidly converted to this compound by the
accessory genital organs of the male rat. One hour after the administration of
[3H] testosterone in vivo, about 70% of the total radioactivity in the prostate
is represented by [3H] 5α-dihydrotestosterone (Bruchovsky & Wilson 1968a,b;
nuclei of the rat ventral prostate can selectively accumulate and retain 5α-

This work was supported by grants from the Norwegian Cancer Society.
dihydrotestosterone in vivo. The same selective retention of 5α-dihydrotestosterone is also found by incubation with [3H] testosterone in vitro. The selective uptake and retention of androgen in the rat prostate is probably due to the interaction of 5α-dihydrotestosterone with specific androgenic receptors. Such binding sites have been found both in the prostatic cytosol (Unhjem et al. 1969) and in the prostatic nuclei (Bruchovsky & Wilson 1968b).

17α-Methyl-β-nortestosterone (SK & F 7690) is a synthetic steroidal compound which inhibits the action of androgens on the seminal vesicles, the ventral prostate and the levator ani muscle in intact and castrated rodents (Saunders et al. 1964). Previous studies have shown that this anti-androgen is able to reduce the uptake of androgen by the prostate in vivo (Tveter & Aakvaag 1969). There are at least two possible explanations for this effect of 17α-methyl-β-nortestosterone. Firstly, the anti-androgen might inhibit the binding of 5α-dihydrotestosterone to androgenic receptors. Secondly, the metabolic conversion of testosterone to 5α-dihydrotestosterone might be interfered with. The purpose of the present work was to investigate the first of these possibilities by studying the effect of this anti-androgenic compound on the association of 5α-dihydrotestosterone with androphilic macromolecules in the rat ventral prostate in vitro.

**MATERIALS AND METHODS**

Male rats, 4 months old, were castrated 28 h before the experiments. They were sacrificed by cervical dislocation. A total of 18 animals were used, 9 of these serving as control animals. Each experiment was performed on pooled tissue from 3 rats. The ventral prostates were dissected free of enveloping fasciae, and then removed and sliced. The slices were incubated in 5 ml of Eagle's tissue culture medium (Eagle 1959), containing 0.0108 µg ([1,2-3H] 5α-dihydrotestosterone (obtained from the New England Nuclear Corporation; specific activity of 46.5 Ci/mmol), for 1 h at 37°C in an atmosphere of air. Before incubation, 7.0 µg 17α-methyl-β-nortestosterone in 35 µl of ethanol, or 35 µl of the vehicle alone (controls), was added to the medium.

After incubation, the slices were homogenized in 9 ml of 0.32 M sucrose containing 1 mM CaCl₂, pH 7.4, using a Potter Elvichem homogenizer. The homogenates were centrifuged at 600 × g for 10 min (3°C) to obtain a crude nuclear fraction. The supernatant fraction was centrifuged at 105 000 × g for 1 h at 3°C. The nuclear fraction was washed twice with 9 ml of the sucrose medium. The final nuclear pellet was suspended in 4 ml of 0.1 M Tris-HCl buffer, pH 7.4. Aliquots were taken for the determination of radioactivity as described previously (Unhjem & Tveter 1969), and for the measurement of protein. The suspension was then made 1 M in NaCl and agitated for 30 min, at 0°C. The extract was centrifuged at 20 000 × g for 15 min (3°C) in order to prepare a supernatant fraction. The supernatant fractions were subjected to gel filtration on a column of Sephadex G-100 (1.9 × 25 cm), at room temperature.

**Acta endocr. 66, 2**
RESULTS AND DISCUSSION

In the control animals, the average uptake of \(^{3}\text{H}\) 5α-dihydrotestosterone by the nuclear fraction was 1235 cPM/mg protein, the values in 3 different experiments being: 1292, 1175 and 1240 cPM/mg protein, respectively. When the incubation medium contained the anti-androgen, the corresponding value was 740 cPM/mg protein (563, 863 and 795 cPM/mg protein). Thus, 17α-methyl-β-nortestosterone reduced the nuclear uptake by about 40%. The prostatic nuclear fraction contained NaCl-extractable macromolecules capable of binding 5α-dihydrotestosterone, as demonstrated previously (Bruchovsky & Wilson 1968b; Mainwaring 1969; Unhjem 1970). These macromolecules were slightly retained by Sephadex G-100 gel. 17α-Methyl-β-nortestosterone seemed capable of interfering with the binding of 5α-dihydrotestosterone to nuclear receptor proteins. Under the conditions used, the anti-androgen almost completely inhibited the formation of the androgen-protein complex (Fig. 1).

The prostatic cytosol contained androphilic macromolecules, in agreement with previous reports (Unhjem & Tveter 1969; Unhjem et al. 1969). In the 105 000 \(\times \) g supernatant fraction of homogenized prostatic tissue, \(^{3}\text{H}\) 5α-dihydrotestosterone was associated with macromolecules excluded from Sep-
hadex G-100 gel. The addition of 17α-methyl-β-nortestosterone to the incubation medium, however, markedly reduced the binding of 5α-dihydrotestosterone to these macromolecules (Fig. 2).

The significance of the present findings in relation to the antagonistic action of 17α-methyl-β-nortestosterone on the prostate, is not clear. The results indicate, however, that this compound interferes with the capacity of an androgen target organ to accumulate and bind 5α-dihydrotestosterone in vitro, suggesting that the anti-androgen may act directly on the prostate. Another anti-androgenic compound, cyproterone, has a similar effect on the uptake and binding of androgen both in the ventral prostate (Fang & Liao 1969) and the seminal vesicles (Stern & Eisenfeld 1969) of the rat. The latter investigators found that cyproterone had no effect on the transformation of testosterone to 5α-dihydrotestosterone. Their results therefore indicate that cyproterone antagonizes the action of androgens by competing for androgenic receptors in a male sexual organ. In order to gain further information on the mode of action of 17α-methyl-β-nortestosterone, it would also be of interest to study its effect on the metabolic conversion of testosterone.

In the present study, the amount of 17α-methyl-β-nortestosterone in the incubation medium was considerable as compared with the amount of 5α-dihydrotestosterone. To obtain more exact data on the ability of this anti-

![Graph](image)

**Fig. 2.**
Gel filtration chromatography of cytosol from the pooled ventral prostate tissue of three castrated rats. The column was equilibrated and eluted with 0.1 m Tris-HCl buffer, pH 7.4. 3 ml fractions were collected. The ordinate indicates cpm in 0.5 ml of the fractions; ----- control; --------- with 17α-methyl-β-nortestosterone.
androgen to inhibit the formation of androgen-receptor complexes, various doses should be tested. Such investigations may profitably be performed both in vivo and in vitro.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr. Olav Unhjem for his kind cooperation in the present work.

17α-Methyl-β-nortestosterone was kindly supplied by Smith, Kline & French Overseas Co., Philadelphia, Pa.

REFERENCES


Received on June 18th, 1970.