ANDROGEN RECEPTOR PROTEIN BINDING PROPERTIES AND TISSUE DISTRIBUTION OF 2-SELENA-A-NOR-5α-ANDROSTAN-17β-OL IN THE RAT.

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ABSTRACT

2-selena-A-nor-5α-androstan-17β-ol was studied in vitro and in vivo in the rat prostate gland. The data demonstrates the ability of this compound to selectively complex with the specific receptors of 5α-dihydrotestosterone (5α-DHT) in the cytosol and to be retained in the nuclei in an unaltered form. Studies with selenium-75 labeled material suggests that the uptake and localization is similar to endogenous 5α-dihydrotestosterone.

INTRODUCTION

Studies with rat prostate minces incubated in vitro have shown that certain structurally modified steroids can inhibit the specific binding of 5α-DHT-^{3}H to androgen receptor protein (1, 2). These studies have emphasized the importance of the 17β-hydroxyl and planarity of the steroid nucleus for maximum binding affinity. In addition, in vivo studies have shown 17β-hydroxyandrostanes lacking the C-3 oxygen retain significant androgenic activity. For example, 5α-androst-2-en-17β-ol (I) was found to have 50 percent of the androgenic activity of testosterone when compared after subcutaneous administration to rats (3). Moreover, the sulfur and selenium

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bioisosteres (II and III) were also shown to have significant androgenic activity (4, 5).

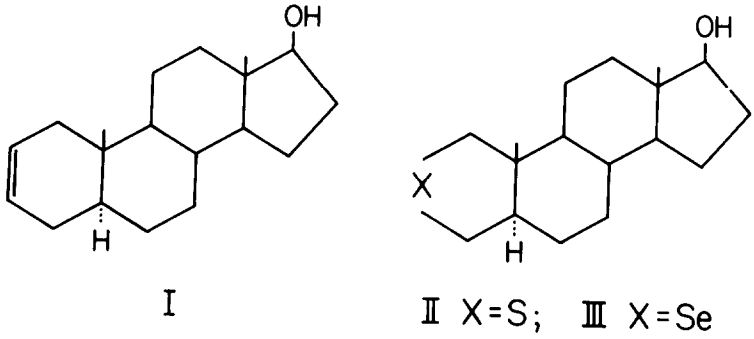

FIGURE I 3-DEOXY-5α-ANDROSTAN-17β-OL DERIVATIVES

Our interest in finding an agent capable of imaging the prostate gland drew our attention to III, since such a compound could be radiolabeled with the gamma-emitting nuclide, selenium-75. Accordingly, this report investigates the ability of 2-selena-A-nor-5α-androstan-17β-ol to compete with 5α-DHT-^{3}H for binding sites in prostate tissue both in vitro and in vivo.

MATERIALS AND METHODS

Materials: Sprague Dawley rats weighing between 300-350 grams were used throughout the study. Tritiated 17β-hydroxy-5α-androstan-3-one (5α-DHT), with a specific activity of 107 Curies/millimole, was purchased from the Amersham/Searle Corporation. 2-selena-A-nor-5α-androstan-17β-ol (III) was synthesized by slight modification of the procedure of Wolff and coworkers (4, 5). The ^{75}Se derivative, (^{75}Se-III) with a specific activity of 3 Curies/millimole, was prepared by the Amersham Radiochemical Center, through the generous auspices of Dr. Allen Peacegood. The homogeneity of these steroids was established by silica gel-HR t.l.c. in the solvent systems, n-hexane:diethylether:acetic acid (86:10:4) and chloroform:methanol:water (90:10:1).

Methods: Rats were castrated via a scrotal incision 24 hours prior to experimentation. 5α-DHT-^{3}H and III were administered as a bolus to ether anesthetized rats via the jugular vein. The vein was exposed by a cut down procedure, with the incision to the right laterally and towards the anterior end of the sternum. Control rats were treated similarly and injected with 5α-DHT-^{3}H. Dosage of III varied with the experimental design. The dose of 5α-DHT-^{3}H was 0.077 microcuries or 0.443 nanomoles per gram of body weight.

Rats were injected, sutured and kept alive without the anesthetic for a determined length of time. The animals were sacrificed and the prostate glands removed. The methods employed in tissue preparation, supernatant dialysis and nuclear purification have been previously published (2). Experiments with ^{75}Se-III were performed by exactly the same procedures except that tritiated 5α-DHT was absent and the radioactive content of the various samples was determined in a Nuclear Chicago Model 4222 Autogamma Counter.

Gel-Filtration: A Sephadex G-200 column (0.9 x 30.0 cm.) was prepared and equilibrated with a 0.01M Tris HCl pH 7.0 buffer, that contained 0.05 mM EDTA, 5.0 mM $MgCl_2$ and 0.5 mM mercapto-ethanol. Approximately 1.5 ml (3.0 mg protein) of the 100,000 x g supernatant was placed on the column and 2.0 ml samples of the eluant were collected. Protein elution was monitored at 280 nm and aliquots of each fraction were removed for radioactivity determination.

Extraction and Thin-layer Chromatography: The extraction of the ^{75}Se radioactivity from the nuclei and supernatant was accomplished by the Folch method (6). The chloroform and methanol-water phases from the extraction were concentrated by rotary evaporation. Aliquots of both phases were counted for radioactivity. The chloroform phases of the supernatant and nuclei were spotted on a t.l.c. plate of silica gel-H.R., 0.5 mm thick. The plate was developed in a system of n-hexane:diethylether:acetic acid (86:10:4). Iodine vapor was employed for visualization.

Tissue Distribution: Various tissues were removed from the rats injected with ^{75}Se-III and placed in beakers containing 1.0 ml isotonic saline. Immediately after dissection, the tissues were blotted and samples weighing between 50-150 mg. were counted in a Nuclear Chicago Autogamma Counter.

RESULTS AND DISCUSSION

Supernatant and Nuclear Inhibition

At a concentration of 1.67×10^{-6} M, III produced 43.0 percent inhibition of 5α-DHT-^{3}H binding in the nuclei and 37.0 percent inhibition in the dialyzed supernatant when a rat prostate mince was used.

The effect of III and 5α-DHT on the *in vivo* binding of radioactive 5α-DHT in the supernatant and nuclei was accomplished by comparing the relative inhibitory responses to these steroids at varying concentrations (0.03 to 3.44 micromoles) (Figure II).

The data indicated both compounds reached saturation levels of inhibition and that 5α-DHT displayed a 12-16 fold greater relative binding potential. The latter observation was accomplished by comparing the micromolar dose of the steroids that would produce 50 percent inhibition. Extensions of the curves of 5α-DHT from the first points to the origin indicated that 50 percent inhibition would occur at approximately 0.02 micromoles. Fifty percent inhibition with III was observed at an injected dose of 0.33 micromoles.

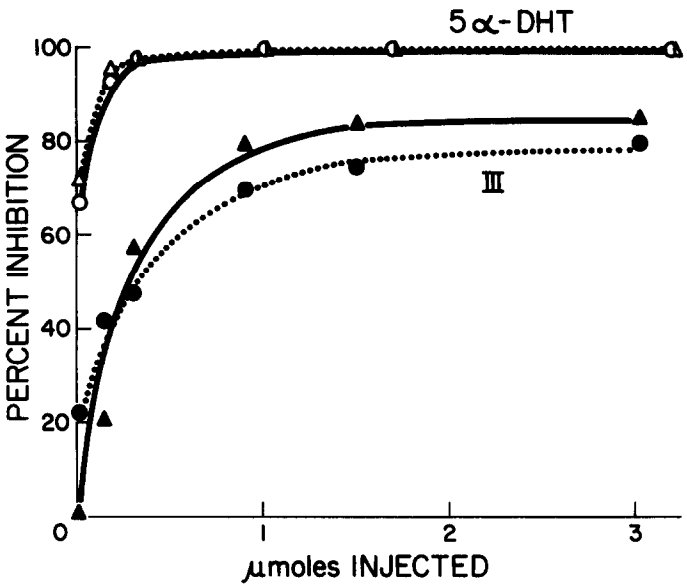

FIGURE II - INHIBITION OF 5α-DHT-^{3}H WITH 5α-DHT AND III IN RAT PROSTATE.

Each steroid was separately combined with 5α-DHT-^{3}H and administered to rats intravenously. After 30 minutes the rats were sacrificed, the prostate removed and the nuclei and supernatant fractions processed as described under methods. The percentages of inhibition of the unlabeled steroids were obtained by comparison to control values in which rats were injected with 5α-DHT-^{3}H only. (△•••△ and ○—○) 5α-DHT supernatant and nuclei; (●•••● and ▲—▲) III supernatant and nuclei.

Evidence of Specific Binding to 5α-DHT Receptors

To further define the observed in vivo inhibitory activity of III, a series of gel-filtration experiments were performed on the dialyzed supernatant.

In the presence of III, the radioactivity of 5α-DHT-^{3}H that was associated with the protein peak in the control supernatant was depressed to background levels. In addition, when dialyzed super-

natant containing ^{75}Se-III was applied on the column, a radioactive protein profile similar to the control supernatant was obtained.

These results provide direct evidence which suggests the inhibitory action of III is a result of competitive binding to 5α-DHT receptor protein in the prostate supernatant. In order to analyze the radioactive material bound to the dialyzed supernatant protein and nuclei after ^{75}Se-III injection, these fractions were isolated and each was extracted as described in the methods. In both nuclei and 100,000 x g supernatant 100 percent of the radioactivity was extracted into the chloroform phase. Thin-layer chromatographic analysis of the chloroform extract indicated that 64 and 67 percent of the radioactivity in the supernatant and nuclei respectively had the same mobility as ^{75}Se-III standard (Rf 0.2). The remaining radioactivity was located at or near the origin and was not characterized.

These results in combination with the gel-filtration experiments suggest that ^{75}Se-III is taken up by the prostate and complexed with 5α-DHT receptors in the supernatant and nuclei mostly in an unaltered form.

Tissue Distribution and Uptake of ^{75}Se-III

The tissue distribution studies (Table I) produced some evidence of selective localization in androgen dependent tissue.

Table I
Tissue Distribution of ^{75}Se-III
CPM/mg. Tissue ± S.E.M.

	10 minutes	1.0 hour	2.0 hours	4.0 hours
VENTRAL PROSTATE	15.4±0.8	22.2±1.8	15.6±3.2	9.4±0.6
DORSOLATERAL PROSTATE	20.3±0.8	14.6±0.8	10.2±1.6	5.8±0.8
SEMINAL VESICLE	24.8±2.7	15.1±1.1	8.1±0.2	4.4±0.6
PITUITARY	57.5±5.2	20.4±1.2	11.8±1.5	7.1±1.1
BLOOD	15.8±0.5	8.6±0.6	8.3±1.4	7.3±0.6
MUSCLE	33.4±1.8	9.9±0.6	4.8±1.1	2.7±0.1
LIVER	213.6+10.2	102.6±3.8	52.6±10.2	54.6±12.7
LUNG	137.3±29.2	113.1±33.4	33.8±2.0	37.6±12.7
ADRENAL	123.8±10.8	36.1±1.9	23.2±3.8	15.0±1.7
THYROID	61.8±7.4	59.4±15.9	77.2±18.3	55.4±7.1
FAT	13.2±1.2	22.5±0.7	28.3±7.8	24.1±3.7

Three rats at each time period were injected with 0.05μCi/gm. body weight or 0.015 nanomoles/gm. body weight. Correction for decay of ^{75}Se was included in each experiment.

The radioactivity in all tissues and the blood, with the exception of the ventral prostate, fat, and thyroid decreased with time. The uptake of ^{75}Se-III by fat tissue is understandable because of the lipid solubility of steroids. However, the uptake and retention of radioactivity by the thyroid was unexpected. Distribution studies at 24 and 48 hours post injection also reflected the same amount of radioactivity as shown by the 4 hour study of the thyroid. A proper explanation for this observation is lacking at the present time.

Since the maximum concentration of ^{75}Se-III in the ventral prostate was observed at one hour after injection, a comparison

experiment was performed with 5α-DHT-^{3}H. The ventral lobes of the prostate were removed from rats injected with 0.08μCi/gm body weight of 5α-DHT-^{3}H at various time intervals up to 2 hours post injection. Comparison of the data indicated the rate of incorporation of 5α-DHT-^{3}H was similar to that of ^{75}Se-III. A similar study has shown that testosterone also reaches maximum concentration in the rat prostate at 1.0 hour (7).

These studies failed to demonstrate the degree of preferential uptake in the prostate that has been observed with tritiated 5α-DHT and testosterone (8, 9). The relatively low specific activity of ^{75}Se-III (3Ci/millimole) compared to that for commerically available 5α-DHT-^{3}H (40-100 Ci/millimole) is believed to be the cause of this failure. Due to the high affinity and low capacity of protein receptors, attempts to observe selective localization of testosterone have failed when low specific activity material was employed (10-15).

In summary, the results of this study demonstrate the feasibility of attaching a gamma-emitting nuclide to a steroid molecule, while still retaining some of the characteristic properties of an endogenous steroid. III was bound to the specific protein receptors of 5α-DHT in the prostatic cytosol and retained by the nuclei. There are also indications that the uptake pattern and localization of the analogue are similar to 5α-DHT. It appears that ^{75}Se-III of high specific

activity could demonstrate the feasibility of using appropriately radiolabeled androgens as prostatic imaging agents.

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