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Androgen Accumulation and Binding to Macromolecules in Seminal Vesicles: Inhibition by Cyproterone

Abstract. *Cyproterone reduces the accumulation of testosterone and dihydrotestosterone in seminal vesicles 30 minutes after intravenous administration of tritiated testosterone to castrated rats. Testosterone, added in vitro, binds to macromolecules from the supernatant fraction of the seminal vesicle homogenates; this interaction is antagonized competitively by cyproterone. Cyproterone may diminish androgenic effects by competition for binding molecules.*

Cyproterone is an experimental steroid (*1*) which antagonizes the effects of androgens in both central and peripheral tissues involved in reproduction. Cyproterone reduced the increase in the weight of the seminal vesicle, prostate, and testis induced by testosterone in hypophysectomized rats (*2*). This inhibition was classified as competitive because the cyproterone effect was greater with lower doses of testosterone (*2*). Androgen secretion by the testis is thought to be responsible for most aspects of sexual differentiation during fetal and early neonatal life (*3*). Cyproterone treatment of rats during pregnancy resulted in male (XY) offspring with a vagina and nipples and the potential for mammary gland enlargement (*4*). The vagina in these males was retained in adulthood and was responsive to estrogen if cyproterone treatment was continued during the first 3 weeks after birth (*5*). Feminization of the hypothalamic regulation of gonadotrophin secretion occurred as well; males exposed to cyproterone as fetuses and newborns can potentially ovulate as evidenced by the development of corpora lutea in transplanted ovaries (*6*). Cyproterone implanted in the basal hypothalamus of intact, sexually mature male rats resulted in hypertrophy of the seminal vesicles, prostates, and testes; apparently, cyproterone blocked feedback inhibition by testosterone of gonadotrophin secretion, leading to increased testosterone amounts (*7*). Cyproterone may compete with testosterone for receptor sites in target tissues. By in vivo and in vitro techniques our findings provide evidence consistent with such a mechanism.

We studied the effects of cyproterone on the accumulation of ^{3}H-testosterone and its metabolites in the seminal vesicle in vivo. After ^{3}H-testosterone is systemically administered, radioactivity is accumulated selectively in both the seminal vesicle and prostate (*8*, *9*). Furthermore, testosterone is rapidly converted in the prostate to a metabolite that has been identified as dihydrotestosterone (5-α-androstan-17-β-ol-3-one) (*10*).

Adult male Sprague-Dawley rats castrated 3 weeks previously were injected intravenously with 0.1 μg of ^{3}H-testosterone (*11*) in aqueous solution per 100 g of body weight. Five minutes before they received the radioactive testosterone, seven experimental males were injected intravenously with cyproterone (1 mg per 100 g of body weight); seven control males received the ethanol vehicle alone. One-half hour later, the tissues were removed, weighed, and rapidly frozen. The tissue was homogenized in 10 ml of acetone, with ^{14}C-testosterone and ^{14}C-androstenedione added to monitor recovery. After 15 to 24 hours at 4°C, the homogenate was filtered, concentrated under a nitrogen stream to about 1 ml, and partitioned twice between 10 ml of ether and 5 ml of H_2O to remove conjugates. After the ether phase was dried, the sample was dissolved in 0.05 ml of methanol, with nonradioactive testosterone and androstenedione added as carriers, for chromatography. The descending chromatographic system consisted of Whatman No. 1 paper impregnated with a mixture of methanol and propylene glycol (60 : 40) as stationary phase and ligroin (b.p. 60° to 90°C) as mobile phase (*12*). Testosterone moves 5 cm from the origin during 24 hours; the mobility of other androgens relative to testosterone in this system is: etiocholanolone, 2.4; dihydrotestosterone, 3.0; androsterone, 4.1; and androstenedione, 5.0. Reference steroids were localized by ultraviolet absorption and the Zimmerman reaction (*12*). The chromatographic strip was cut into small segments, and radioactivity was determined in a liquid-scintillation counter set for dual isotope counting. The values are corrected for interference in the ^{3}H-channel by ^{14}C, for counter efficiency, and for recovery of the ^{14}C tracer. Further characterization of the identity of the radioactivity in the testosterone and dihydrotestosterone zones was obtained by rechromatography and crystallization to constant specific activity.

Higher concentrations of testosterone and dihydrotestosterone were found in the seminal vesicle than in plasma (Fig. 1). One-half hour after intravenous administration of radioactive testosterone, the concentrations of testosterone and dihydrotestosterone in the seminal vesicles were 3 and 80 times greater, respectively, than those in the plasma. In the seminal vesicle the radioactivity was distributed as 27 percent testosterone, 63 percent dihydrotestosterone, and less than 2 percent androstenedione, the remainder being primarily metabolites more polar than testosterone. Cyproterone reduced the radioactive testosterone and dihydrotestosterone in the seminal vesicle to 20 and 15 percent, respectively, of that in the control concentrations (Fig. 1). The ratio of testosterone to dihydrotestosterone was not significantly different in the control and cyproterone groups ($F = 0.04$; d.f. = 1.12). Concentrations of testosterone and dihydrotestosterone in plasma were unchanged by cyproterone treatment.

Our experiments in vitro indicate that cyproterone may compete with androgens for macromolecules from the seminal vesicle. The seminal vesicles and hearts from ten mature male Sprague-Dawley rats castrated 3 weeks previously were homogenized in 12 volumes of 0.01*M* tris(hydroxymethyl)aminomethane-hydrochloride, *p*H 7.4, containing 0.0015*M* ethylenediaminetetraacetate.

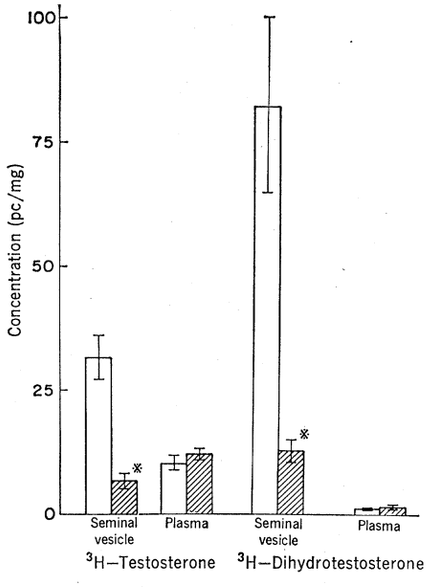

Fig. 1. Radioactive testosterone and dihydrotestosterone concentrations 30 minutes after intravenous administration of ^{3}H-testosterone. Control animals (open bars ± S.E.M.) received ^{3}H-testosterone; experimental group (cross-hatched bars ± S.E.M.) received cyproterone intravenously 5 minutes before the radioactive testosterone. Asterisk, $P < .01$.

The particulate fractions were removed by centrifugation for 10 minutes at 5000*g* followed by centrifugation at 100,000*g* for 1 hour. Portions of the supernatant (0.2 ml) were added to aqueous solutions of ^{3}H-testosterone, so that the final concentrations of ^{3}H-testosterone were 0.5, 2, 10, or $20 \times 10^{-9}M$, with or without $1 \times 10^{-7}M$ cyproterone. After 1 hour in ice, the labeled macromolecule was separated from free radioactivity by gel filtration on small polyacrylamide columns (Biogel P-10). Radioactivity in the macromolecular fraction was then extracted into toluene and a portion counted.

Radioactivity was found in the macromolecular fraction of the supernatant of the seminal vesicle homogenate upon addition in vitro of ^{3}H-testosterone (Fig. 2). Bound to 0.2 ml of the supernatant were 240 dpm (disintegrations per minute) when the final ^{3}H-testosterone concentration was $0.5 \times 10^{-9}M$; 700 dpm with $2 \times 10^{-9}M$; 960 dpm with $10 \times 10^{-9}M$; and 1080 with $20 \times 10^{-9}M$. This radioactive material co-chromatographed with authentic testosterone on silica-gel thin-layer plates with a mixture of chloroform and ethyl acetate (80:20) as solvent (*13*). The calculated testosterone capacity of these binding molecules in the undiluted supernatant of the seminal vesicle homogenates in $0.6 \times 10^{-9}M$. Macromolecular binding of radioactivity could not be demonstrated in a control tissue, the heart. In a predominantly competitive manner, cyproterone reduced the binding of testosterone to the macromolecules in the supernatant of the seminal vesicle homogenate (Fig. 2). The affinity of testosterone for the macromolecules was about 28 times greater than that of cyproterone.

Our results confirm the observation of concentration of testosterone and dihydrotestosterone in an androgen responsive organ (*9, 10*). In contrast to findings reported for the guinea pig (*8*), we found little if any androstenedione or etiocholanolone in the seminal vesicle. After injection of radioactive testosterone, testosterone and even higher concentrations of dihydrotestosterone are found attached to chromatin protein in the prostate (*14*). The conversion appears to be due to a 5α-reductase (3-ketosteriod : nicotinamide adenine dinucleotide phosphate, Δ^4-oxidoreductase; E.C. 1.3.99.5) which is partially associated with prostatic chromatin (*10*). Dihydrotestosterone is known to have the same order of androgenic potency on peripheral target organs as testosterone when either administered systemically or applied locally to the chick comb (*15*).

In general we agree with the report of macromolecular binding of testosterone by the supernatant of the seminal vesicle homogenate (*16*). The demonstration of binding of testosterone to macromolecules from the supernatant of the seminal vesicle homogenate suggests that the initial interaction of androgen and macromolecule may occur in the cytoplasm with subsequent attachment of this complex to chromatin. Evidence for transfer of estrogens from cytoplasmic macromolecules to nucleus has been presented (*17*). Androgens and estrogens have similarities of biochemical action in their respective reproductive target organs, especially with regard to early increases in RNA synthesis (*18*).

Cyproterone prevents accumulation of both testosterone and dihydrotestosterone in the seminal vesicle. Chronic treatment with cyproterone acetate partially decreases radioactivity in the seminal vesicle after injection of ^{3}H-testosterone (*19*). Our finding of an unchanged ratio of dihydrotestosterone to testosterone suggests that cyproterone does not decrease radioactive accumulation by selective direct inhibition of the enzymatic formation of dihydrotestosterone. Our results in vivo and in vitro are consistent with the hypothesis that cyproterone antagonizes the action of testosterone by competing for macromolecules which may be androgenic receptors in a male sexual organ. Inhibition of the interaction of androgen with macromolecules may explain the cyproterone diminution of testosterone-induced increase in RNA synthesis in vitro in prostatic nuclei of castrated rats (*20*).

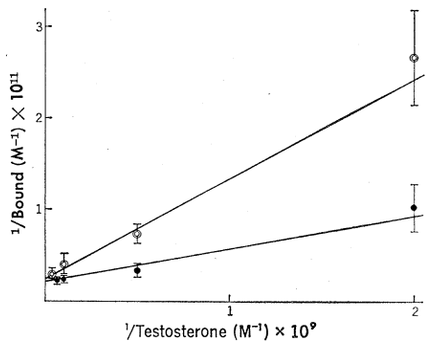

Fig. 2. Double reciprocal plot of ^{3}H-testosterone concentration and radioactivity bound to macromolecules from the seminal vesicle in the presence (open circles ± S.E.M.) and absence (closed circles ± S.E.M.) of $1 \times 10^{-7}M$ cyproterone. Lines were drawn by weighted least-squares fit of the data.

Observations on the hypothalamic regulation of gonadotrophins indicate that cyproterone affects central as well as peripheral tissues involved in reproduction (*6, 7*). In additional studies (*21*), treatment with cyproterone before intravenous administration of ^{3}H-testosterone prevented accumulation of testosterone and dihydrotestosterone in the hypothalamus and pituitary. This supports the concept that modification of androgen effects in the hypothalamus and pituitary by cyproterone may be due to competition for binding molecules.

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Chromosomal Fragments Transmitted through Three Generations in Oncopeltus (Hemiptera)

Abstract. *Chromosomal fragments and translocations induced by x-rays in the sperm of adult milkweed bugs,* Oncopeltus fasciatus *(Dallas), were detected in the meiotic cells of F_1, F_2, and F_3 males and caused high levels of sterility in untreated progeny. The persistence of these fragments through numerous generations of cells confirmed the holokinetic nature of the milkweed bug chromosomes.*

Since the majority of plants and animals possess chromosomes with a single centromere or kinetochore, these species usually lose any chromosomal fragments at the time of cell division because there is no spindle fiber attachment. In some plant and animal species with holokinetic chromosomes (*1*, *2*) chromosome fragments have been observed to persist for several cell generations (*1*, *3*), presumably because all fragments are capable of orientation and anaphase movement during cell division. The present report describes the induction of chromosomal fragments and reciprocal translocations in the sperm of adult milkweed bugs, *Oncopeltus fasciatus* (Dallas), the transmission of these fragments and translocations through three outcrossed generations, and the consequent effects on the fertility of the progeny.

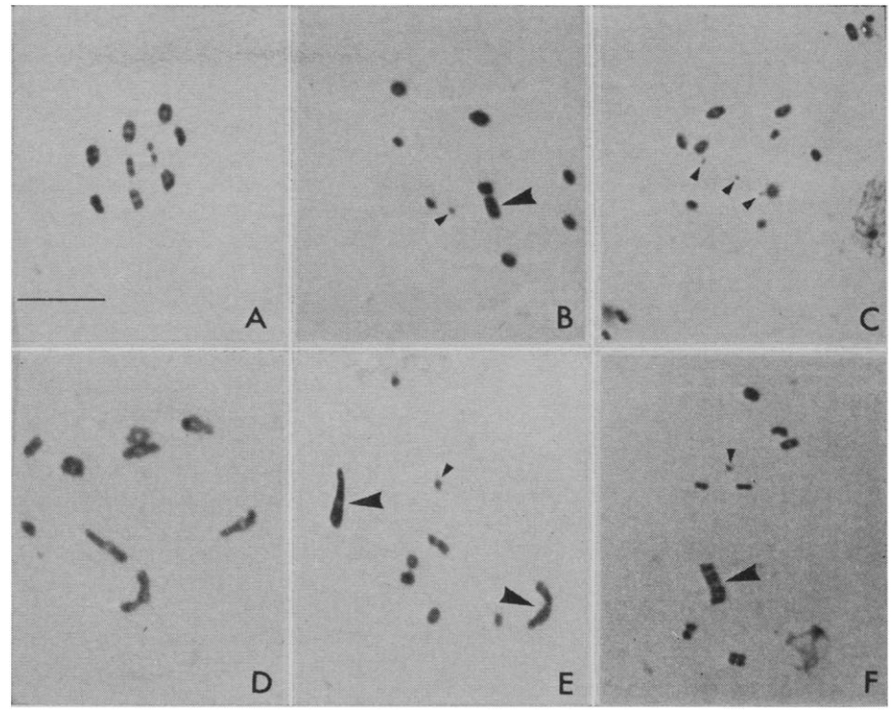

Fig. 1. Chromosomes in the primary spermatocyte of *Oncopeltus*. All magnifications about the same; line in (A) represents 10 μm. (A and D) Normal complement of chromosomes in control males—seven paired bivalents plus X and Y; (B and E) chromosomes in testes of same F_1 male; and (C and F) chromosomes in testes of an F_2 male. Small arrows indicate fragments; large arrows, a heterozygous translocation complex.

Adult males, 3 to 5 days old, were irradiated with 9 kiloroentgens of x-rays and placed in individual cages with one untreated female. Eggs were collected from each of 29 pairs and scored for hatchability. Then the F_1 generation was reared to the adult stage and sexed, and the individual males were outcrossed to virgin untreated females as before. The procedure was repeated until F_2 and F_3 progeny were produced. These repeated outcrossings to untreated females assured that all progeny would contain a normal complement of chromosomes from the female and the fragmented or rearranged set from the male. Also, after a sufficient number of eggs had been collected from each pair, the male was removed, and the testes were dissected, fixed in 45 percent acetic acid, and squashed on a microscope slide. Material on the slide was frozen on a block of Dry Ice, the coverslip was removed, and the testes squash was stained by the Feulgen reaction. Although numerous lines were studied, each originating from a single F_1 male and many exhibiting the persistent transmission of chromosomal fragments and translocations, the data from only a single line are presented. The cytology and fertility of all lines will be reported elsewhere.

One F_1 male derived from an irradiated sperm and an untreated egg had a fertility of 5.8 percent. The extent of chromosomal fragmentation and rearrangement in this male is shown in Fig. 1, B and E, and can be compared with the normal meiotic configurations shown in Fig. 1, A and D. Most sperm produced by this male contained chromosomal duplications or deficiencies, or both, that rendered the sperm incapable of supporting embryonic development. However, three male and eight female F_2 progeny were produced. One of the males was only 2.1