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Intrauterine Position Effects on Steroid Metabolism and Steroid Receptors of Reproductive Organs in Male Mice[1]

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ABSTRACT

Mice differ in their adult reproductive characteristics as a function of whether they developed in utero between two male fetuses (2M males), which have higher testosterone levels, or between two female fetuses (0M males), which have higher estradiol levels. The present study was designed to further characterize biochemical parameters of 2M and 0M adult male mice. Activities of testicular steroidogenic enzymes, namely Δ^5–3β-hydroxysteroid dehydrogenase/isomerase, 17α-hydroxylase, and $C_{17,20}$-lyase (C_{21SCC} P_{450}), were measured by means of radiometric assays and HPLC fractionation of substrate and products. Activity of 5α-reductase in both seminal vesicle and prostate was measured by similar techniques. Estrogen and androgen receptor concentrations, which indicate capacity to respond to steroid hormones, were also examined in the accessory sex organs. For both seminal vesicle and prostate, 5α-reductase activities were approximately 60% greater in 2M males than in 0M males, indicating greater capacity to form dihydrotestosterone from testosterone in organs from 2M mice. No significant differences were found in testicular steroidogenic enzymes between 2M and 0M animals, whereas the trend for all three activities was higher for 2M males than for 0M males. While no differences were found in estrogen receptor concentrations, 0M prostates had three times the concentration of androgen receptors (occupied receptors) compared to 2M prostates. Our findings suggest that intrauterine fetal position exerts a significant influence on subsequent adult androgen metabolism and androgen responsiveness in reproductive organs of adult male mice.

INTRODUCTION

In mice and rats, a significant component of phenotypic variation among adult individuals of the same sex is due to the contiguity of an animal to siblings of the same or opposite sex during intrauterine development [1, 2]. This naturally occurring phenotypic variation due to random arrangement of fetuses is referred to as the intrauterine position phenomenon [2]. Fetal mouse testes differentiate at approximately Day 13 of gestation and begin to secrete testosterone (T) [3], which remains elevated until birth at approximately Day 19 in CF-1 mice [2]. Amniotic fluid and blood of animals that develop between two males (2M) have greater concentrations of T, but lesser concentrations of estradiol, than those of animals that develop between two females (0M); [4–6]. Intrauterine fetal position effects on female development have been studied extensively. Females that develop between two males in utero have a larger anogenital distance than females that develop between two females; i.e., 2M females are slightly masculinized [2]. As adults, 2M female mice are more aggressive, have longer (6-day vs. 4-day) estrous cycles, and cease producing young at an earlier age than do 0M females [4–6].

Intrauterine fetal position also influences male development; however, this has been less extensively described. Gonadally intact 0M males exhibit more mounts and more intromissions when paired with females [6]. When male 2M and 0M mice were castrated at birth to eliminate any postnatal androgen exposure, and then treated with T by implants in adulthood, the 2M males were found to be more aggressive towards other males than were the 0M males [5, 6]. For these reasons, the naturally occurring phenomenon of random intrauterine positioning of male and female fetuses affords a unique model for studying the effects of small physiological differences in fetal hormone concentrations on later development.

In the present study, we investigated certain biochemical parameters that would allow us to further characterize the effects of intrauterine position in adult male mice. We measured activities of three important testicular enzymes to monitor the steroidogenic potential. 5α-Reductase activity was measured in accessory sex tissues (i.e., seminal vesicles and prostate) to determine the ability of 2M and 0M mice to convert T to dihydrotestosterone (DHT), a more potent androgen. In addition, we measured steroid hormone receptors in the accessory sex organs to assess the ability of these tissues to respond to androgens and estrogens.

MATERIALS AND METHODS

Materials

Steroid standards were purchased from Sigma Chemical Co. (St. Louis, MO) and recrystallized from aqueous ethanol prior to use. New England Nuclear (Boston, MA) was the source for [1,2,6,7,16,17-^{3}H]T (168 Ci/mmol), [1,2,4,5,6,7-^{3}H]DHT (148 Ci/mmol), [2,4,6,7-^{3}H]estradiol (106.9 Ci/mmol), [1,2,6,7-^{3}H]progesterone (115 Ci/mmol), [1,2-^{3}H]17α-hydroxyprogesterone (42.3 Ci/mmol), and [7-^{3}H]preg-

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nenolone (10.5 Ci/mmol); their purity was checked by HPLC. Acetonitrile, methanol, benzene, and hexane were HPLC-grade from Burdick and Jackson (Muskegan, MI). Ethanol (HPLC-grade) was purchased from J.T. Baker Chemical Company (Glen Ellyn, IL). β-NADPH, NAD^+, and other reagents were purchased from Sigma.

Animals and Identification of Intrauterine Fetal Position

An outbred colony of CF-1 house mice (*Mus musculus domesticus*), originally purchased from Charles River Laboratories (Wilmington, MA), was used to take advantage of the large litter size (12 pups). The mice were maintained on a 12L:12D cycle. Adult female mice were time-mated and checked for the presence of vaginal plugs (Day 0). On Day 19 of gestation, the mothers were killed by cervical dislocation, and the pups were delivered by Caesarean section within 2 min of maternal death. Fetal sex was assessed by the length of the anogenital space, the intrauterine fetal position of each pup was determined, and each pup was marked by toe clipping for positive identification. A 2M pup was between two males in the uterus, while a 0M pup was between two female fetuses. Pups between a male and female fetus in a uterine horn were not used in these experiments.

The male pups, in groups of five 0M and five 2M, were fostered to females that had delivered normally within the preceding 24 h. The animals were weaned at 23 days and housed individually at 35 days of age. 0M and 2M males were matched for body weight when assigned to the experiments. In adulthood, after 100–115 days of age, animals were killed by cervical dislocation, and the tissues were collected and stored at −70°C until further analyses.

Testicular Steroidogenic Enzyme Assays

Testes were individually homogenized at 10 ml/g in Krebs-Ringer phosphate buffer (KRPB, pH 6.9) prepared as previously described [7] and were held at 0–4°C at all times prior to incubation. Ten milligrams of testicular tissue was placed into incubation flasks containing 0.5 μCi [^{3}H]progesterone and enough nonradioactive progesterone so that the final progesterone concentration equalled 5 μM in a 1.0-ml incubation volume. This mixture was preincubated for 2 min at 37°C before initiation of the reaction via addition of 0.5 mM β-NADPH. The reaction was terminated after 30 min. Assay conditions were specifically designed (for each enzyme) to maintain a linear reaction velocity with respect to time and protein concentration. Product accumulation for all three enzymes was linear through 60 min and 2 mg protein, which were the maximum parameters examined. Control incubations were processed in exactly the same manner with boiled testicular homogenate from each group used. The background activity was below the limits of detection for all control incubations. All incubations were performed in duplicate.

After the incubation, the reaction was terminated by placing the incubation vials on ice. The sample preparation procedure, using C_{18} solid-phase extraction (SPE) columns, was conducted as described and analyzed in detail elsewhere [8]. Briefly, the C_{18} SPE columns were activated by discharging 2.0 ml of methanol followed by 5.0 ml of water through the column with positive air pressure. Incubation vial contents were transferred to the column and washed with 8.0 ml methanol/water (1:3 v/v) and 2.0 ml of *n*-hexane. Steroids were eluted from the column with 4.0 ml of benzene into glass conical tubes. The benzene was evaporated, and the conical tube contents were concentrated to the tip.

The activity of 17α-hydroxylase was calculated by using the value of 5 μM progesterone initially present in each incubation flask and the percentage of the total radioactive metabolites formed, i.e., 17α-hydroxy-progesterone, 4-androstene-3,17-dione, and T. Data are expressed as specific activity in nanomoles of product formed in 30 minutes per milligram of protein. Lyase or side-chain cleavage activity was determined in exactly the same manner except that 17α-[^{3}H]hydroxyprogesterone was used as substrate. The percentage of conversion to 4-androstene-3,17-dione and T was used to calculate specific activity. The enzymatic activity of Δ^5-3β-hydroxysteroid dehydrogenase/isomerase was similarly determined except that [^{3}H]pregnenolone was used as substrate and 0.5 mM β-NAD^+ was used as the cofactor. The percentage of conversion to progesterone was used to calculate specific activity.

Quantification of Steroid Metabolites by Isocratic HPLC

We employed the use of an isocratic ternary solvent system and a narrow-bore reversed-phase HPLC column to accomplish the separation of pregnenolone, progesterone, 17α-hydroxyprogesterone, 4-androstene-3,17-dione, and T. Steroids were judged to be completely separated from each other on the basis of the resolution of adjacent radioactive peak pairs. The resolution between peaks was always greater than 1.25, indicating complete separation of compounds [7].

A Perkin-Elmer (Norwalk, CT) Series 10 liquid chromatograph equipped with a Rainin (Woburn, MA) Rheodyne model 7125 injector, a 100-μl sample loop, a Beckman-Altex (Fullerton, CA) 2 × 250-mm Ultrasphere ODS (5 μm) reversed-phase C_{18} column protected by an Upchurch (Oak Harbor, WA) 2-μm precolumn filter, was used for the chromatography. The elution of 4-ene-3-ketosteroids was monitored through UV absorbance detection at 240 nm; the elution of pregnenolone (a 5-ene-3β-hydroxysteroid) was monitored at 219 nm with a Perkin-Elmer model LC-95 UV/VIS spectrophotometric detector equipped with an 18-μl flow cell. Detection and quantification of ^{3}H-isotope elution was accomplished by use of a Beckman Model 171 on-line radiochromatographic detector equipped with a 300-μl flow cell. An isocratic acetonitrile:methanol:water (35:24:40, v:v:v)

ternary solvent system at a flow rate of 0.3 ml/min (2000 psi) was employed for the 20-min analysis.

5α-Reductase Radiometric Assay

Prostate and seminal vesicle tissues were transferred to polypropylene homogenizing cylinders and homogenized in an amount of KRPB equal to 10 ml/g tissue original wet weight. Homogenization with a type PT 10/35 Polytron (Brinkman Instruments, Westbury, NY) was performed on ice so that tissues remained at 0–4°C. Ten milligrams of tissue was placed into separate incubation vials containing 0.05 μCi [^{3}H]T (100 000 dpm) and enough recrystallized nonradioactive T so that the final T concentration was 5×10^{-9} M in a 1.0-ml incubation volume. The final concentration of β-NADPH, prepared with KRPB in each incubation vial, was 0.5 mM. Incubations were conducted at 22°C for 1 h [8]. Assay conditions for the enzyme reactions were designed to give a substrate conversion rate of not less than 1% and not more than 15% to ensure linearity of the reaction with respect to time and tissue concentration. In order to account for any endogenous reducing ability in the homogenates, control tissues were processed in exactly the same manner except that they did not contain β-NADPH. We did not see any measurable endogenous reducing ability in the tissue homogenate. Our preliminary control experiments using boiled prostatic and seminal vesicular tissue failed to detect 5α-reduced T metabolites (e.g., 5α-androstane-3α-diol [3α], 5α-androstane-3β-diol [3β], and 5α-DHT), suggesting that little spontaneous reduction of T occurs under the above experimental conditions through the 1-h incubation period. A C_{18} SPE steroid elution procedure was used to isolate the androgens from the biological matrix, as described above.

Samples were reconstituted in 30 μl HPLC-grade ethanol containing authentic recrystallized standards T, 3β, 3α, and DHT to aid in the UV visualization at 210 nm. A Rainin 4.6-mm × 25-cm Microsorb ODS reverse-phase C_{18} column (5-μm packing) and a Brownlee (Rainin, Woburn, MA) 3.2-mm × 1.5-cm HPLC C_{18} guard column were used. All other chromatographic conditions were the same as above. An isocratic acetonitrile/water (45:55 v/v) solvent system at a flow rate of 1.0 ml/min (1800 psi) was used for the 20-min analysis. Detection and quantification of ^{3}H-isotope elution was accomplished by use of the Beckman Model 171 on-line radioisotope detector equipped with a 1.0-ml flow cell [9]. The counting efficiency of on-line radiochromatographic detection was 20% when scintillation cocktail (Scintiverse LC; Fisher Scientific, Pittsburgh, PA) was mixed with HPLC effluent (3:1 ratio) in a high-efficiency mixer. The formation of 5α-reduced androgens was calculated with use of the value of 5×10^{-9} M T initially present in the incubation and the percentage of total radioactive 3β, 3α, and DHT formed.

Estrogen Receptor Assay

Estrogen receptors were measured by the hydroxylapatite (HAP) method of Williams and Gorski [10]. Briefly, tissues were excised and placed immediately on ice. Tissues were homogenized in 10 mM Tris-HCl, 1.5 mM sodium-EDTA, and 1 mM dithiothreitol (pH 7.3, 25°C) (TED) at a ratio of 1:20 (w/v). Aliquots of the cytosol (100 000 × *g* for 1 h) containing 0.5–2.0 mg/ml of protein were incubated with 1–10 nM [^{3}H]estradiol overnight at 4°C. Nonspecific binding was determined by using 100-fold excess of nonradiolabeled steroid in parallel incubations. To separate bound from free hormone, 200 μl of cytosol was added to a 60% slurry of HAP in 50 mM Tris + 1 mM KH_2PO_4 and allowed to stand on ice with several mixings for 30 min. The HAP pellet was washed five times in 50 mM Tris-HCl, pH 7.3. After the final wash, radioactivity was extracted from the pellet with 2 ml of ethanol and counted in 10 ml of scintillation cocktail.

Androgen Receptor Assay

Tissues were immersed in ice-cold TEDG buffer (10 mM Tris, 1.5 mM EDTA, 10% glycerol [v/v], and 1 mM each of dithiothreitol, phenylmethylsulfonyl fluoride, and sodium molybdate; pH 7.4) [11]. Tissue was homogenized on ice (1 g tissue/5 ml buffer) with 5-sec bursts of a polytron. The homogenate was centrifuged at 30 000 × *g* for 30 min. This supernatant was used as the low-salt extract and contained the unoccupied cytosolic receptors [12]. The pellet was homogenized every 15 min for 1 h in high-salt buffer (TEDG buffer with 0.4 M KCl) and centrifuged as before. This supernatant was used as the high-salt extract and contained receptors that were occupied by endogenous hormone [12].

Before incubation with labeled hormone for the binding assay, endogenous steroids were removed from the low-salt extract by incubation with dextran-coated charcoal (DCC; 12.5 mg charcoal plus 1.25 mg dextran/ml of extract). After 1 h at 0–4°C, DCC was removed by centrifugation at 10 000 × *g* for 10 min. Specific binding of [^{3}H]DHT by the extracts was estimated by competitive inhibition using nonlabeled DHT. An aliquot of extract (550 μl) was incubated with 0.5–20.0 nM [^{3}H]DHT. Parallel tubes contained 100-fold excess nonradioactive DHT to determine nonspecific binding. The extracts were incubated for 24 h at 4°C, during which labeled DHT bound to the unoccupied receptors in the low-salt extract or exchanged with endogenous hormone bound to the occupied receptors in the high-salt extract. Free steroid was separated from bound steroid by adding, in duplicate, 200 μl of extract to 0.3–0.35 ml of packed HAP in 0.5 ml buffer and incubating this mixture for 20 min with several mixings. The samples were then centrifuged for 2 min at 600 × *g* and 4°C. The supernatant was aspirated, the packed HAP was washed four times with 2 ml ice-cold 10 mM Tris buffer (pH 7.3), and bound hormone was extracted with ethanol as described above.

TABLE 1. Effects of intrauterine position on testicular steroidogenic enzyme activities.*

Enzyme	0M	2M
Δ^5-3β-Hydroxysteroid Dehydrogenase/isomerase	7.68 ± 0.64	7.89 ± 0.89
17α-Hydroxylase	1.34 ± 0.11	1.60 ± 0.20
$C_{17,20}$-Lyase	2.54 ± 0.13	2.62 ± 0.31

*Data are expressed as nmol product formed/30 min/mg protein (mean ± SEM, n = 7 testes/group). The designations 0M and 2M represent pups neighbored in utero by two females or two males, respectively.

Protein Determination

Protein determinations of tissue homogenates were carried out according to Bradford [13]. Briefly, homogenates were digested with 0.1 N NaOH at 60°C for 1 h, diluted in Tris buffer (pH 7.5), and assayed in duplicate for protein concentration against a standard curve of 5–50 μg/ml of BSA.

Data Analysis

Data were expressed as enzyme activity or receptor number per milligram protein. Data for enzyme activities and receptor numbers were subjected to one-way ANOVA. For all organ weights, body weight was used as the covariate in covariance analysis. The level of significance was set at $p < 0.05$.

RESULTS

Testicular Steroidogenic Enzymes

We examined the activities of three enzymes, namely Δ^5-3β-hydroxysteroid dehydrogenase/isomerase, 17α-hydroxylase, and $C_{17,20}$-lyase. Activities were determined radiometrically and are expressed as specific activity in nanomoles of product formed per milligram of protein. Since the testis weights were not different between 2M and 0M adult mice (123.3 ± 5.3 vs. 121.2 ± 3.0 mg, respectively) total activity paralleled the values seen for specific activity.

Δ^5-3β-Hydroxysteroid Dehydrogenase/Isomerase

With pregnenolone as substrate and NAD^+ as the cofactor, progesterone was the single product detected when Δ^5-3β-hydroxysteroid dehydrogenase/isomerase activity was measured in whole testicular homogenates. No significant differences were seen between 2M and 0M adult mice (Table 1).

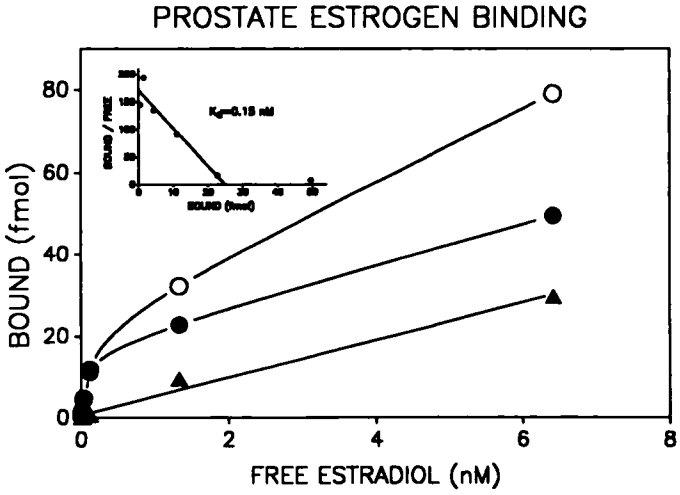

FIG. 1. Total (○), nonspecific (▲), and specific binding (●, the difference between total and nonspecific) of 17β-[^{3}H]estradiol to estrogen receptors from mouse prostate cytosol. Cytosols were incubated for 22 h at 4°C with the indicated concentrations of 17β-[^{3}H]estradiol. Nonspecific binding was determined by parallel incubations containing 100-fold excess of estradiol. Inset, Scatchard plot of estrogen binding in the presence of 10 nM DHT (to saturate androgen binding) in prostates from gonadally intact 0M and 2M male CF-1 mice.

17α-Hydroxylase and $C_{17,20}$-Lyase Activity

The conversion of progesterone to androstenedione occurs through two activities of one testicular enzyme [14]. Both activities were measured in 0M and 2M testicular homogenates to evaluate their ability to form androgens from progesterone (Table 1). With progesterone as substrate and NADPH as the cofactor for 17α-hydroxylase activity, whole homogenates converted about 1.5 nmol of progesterone to 17α-hydroxyprogesterone and androstenedione per milligram of protein, with 17α-hydroxyprogesterone predominating throughout 17α-hydroxylase activity. In the 30-min incubation, $C_{17,20}$-lyase activity yielded an average of 2.6 nmol of C_{19}-steroids from 17α-hydroxyprogesterone.

5α-Reductase Activity

Seminal vesicle. At 5×10^{-9} M T, conversion rates of 2–5% were obtained under the conditions described in *Materials and Methods*. Seminal vesicles of 2M mice were

TABLE 2. Effects of intrauterine position on reproductive organ weights and 5α-reductase activity.*

	Seminal vesicle		Prostate	
	0M	2M	0M	2M
Weight (mg)	85.0 ± 10.4	131.8 ± 9.01*	59.5 ± 3.3	49.4 ± 3.0*
5α-Reductase activity	0.17 ± 0.03	0.25 ± 0.04*	0.19 ± 0.02	0.29 ± 0.04*

*Data are expressed as pmol product formed/h/mg protein (mean ± SEM, n = 7 tissues/group). The designations 0M and 2M represent pups neighbored in utero by two females or two males, respectively.
*Significantly different, $p < 0.05$.

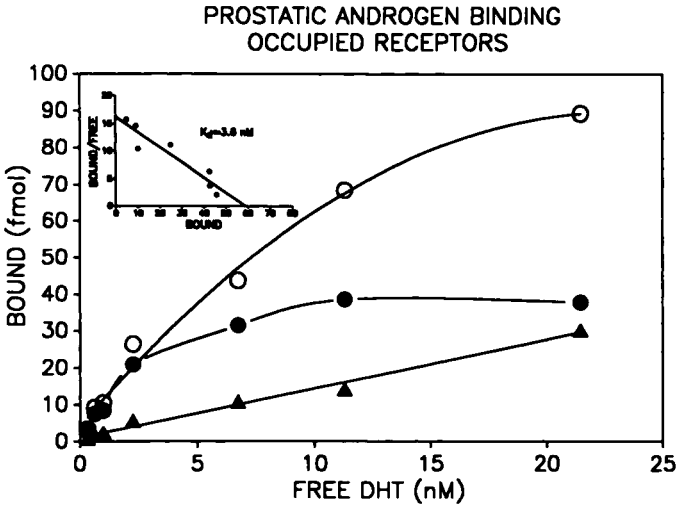

FIG. 2. Total(○), nonspecific (▲), and specific (●) binding of [^{3}H]DHT in high-salt extracts from mouse prostate. High-salt extracts were incubated for 22 h at 4°C with the indicated concentrations of [^{3}H]DHT. Nonspecific binding was determined by parallel incubations containing 100-fold excess of DHT. Inset, Scatchard plot of androgen binding in prostates from gonadally intact 0M and 2M male CF-1 mice.

significantly heavier and had higher 5α-reductase activity ($p < 0.05$) than seminal vesicles of 0M mice (Table 2). Analysis of covariance showed that body weights accounted for virtually none of the variance ($F = 0.1, p = 0.73$) in seminal vesicle weight.

Prostate. The mean specific activity of 5α-reductase was significantly greater ($p < 0.05$) in prostates from 2M males than from 0M males (Table 2). The specific activity of 5α-reductase ranged from 0.195 to 0.428 pmol/h/mg protein in 2M males and from 0.079 to 0.267 pmol/h/mg protein in 0M males. Prostatic weight, however, varied significantly ($p < 0.05$) as a function of intrauterine position in that the 0M prostates were heavier than prostates obtained from 2M animals (Table 2). This difference was not due to variance in body weight ($F = 0.7, p = 0.45$). Total 5α-reductase activity was not different since 0M prostates were heavier than 2M prostates.

Estrogen Receptor Studies

Specific estrogen receptors were found in cytosols prepared from mouse prostate, whereas cytosols prepared from seminal vesicles exhibited very low binding of 17β-[^{3}H]estradiol. Estrogen binding studies using mouse prostate cytosols showed saturable binding by 2 nM estradiol. Scatchard analysis revealed two components: a high-affinity component with low capacity and a lower-affinity component. The addition of 10 nM DHT (to reduce any cross-binding of labeled estrogen to androgen receptors) reduced the lower-affinity component. The main component showed high-affinity binding and low capacity: $K_d = 0.15$ nM and $B_{max} = 30$ fmol/mg protein (Fig. 1). Competition studies carried out as previously described [15], i.e. using 100-fold excess of estradiol, estriol, T, DHT, progesterone, and cortisol against 2 nM labeled estradiol showed that, in addition to estrogens, only DHT competed with binding of estradiol.

Estrogen binding assays were performed on prostatic tissue in duplicate, with 2 nM [^{3}H]estradiol in the presence of nonradioactive 10 nM DHT. Prostate estrogen receptor concentrations expressed as fmol/mg protein were not significantly different ($p > 0.05$) between 0M and 2M mice (18.06 ± 1.80 vs. 15.77 ± 2.00 fmol/mg protein, respectively; n = 7 per group).

Androgen Receptor Studies

Androgen binding was demonstrated in both high-salt and low-salt extracts (occupied and unoccupied receptors, respectively) of mouse prostate and seminal vesicle. Approximate saturation was reached near 20 nM DHT (Fig. 2). The observed affinity of DHT binding ($K_d = 3.6$ nM) was comparable to values previously reported [16, 17]. Time course incubations showed that androgen binding was maximal from 8 to 24 h of incubation at 4°C. Competition studies using DHT, T, estradiol, progesterone, and cortisol showed that DHT and T were the most effective competitors for binding in the mouse prostate. However, a 100-fold excess of estradiol competed partially with DHT for binding. Low-salt extracts (unoccupied receptors) of the seminal vesicle and prostate gave low binding values, so only high-salt extracts (occupied receptors) were used for comparisons of androgen receptor concentrations in these tissues from 0M and 2M males.

Androgen binding in the extracts of 0M and 2M seminal vesicles did not differ significantly ($p > 0.05$) at 15.30 ± 7.52 vs. 16.90 ± 4.33 fmol/mg protein, respectively (n = 7/group). However, in the prostate, androgen receptors in high-salt extracts (occupied receptors) were significantly

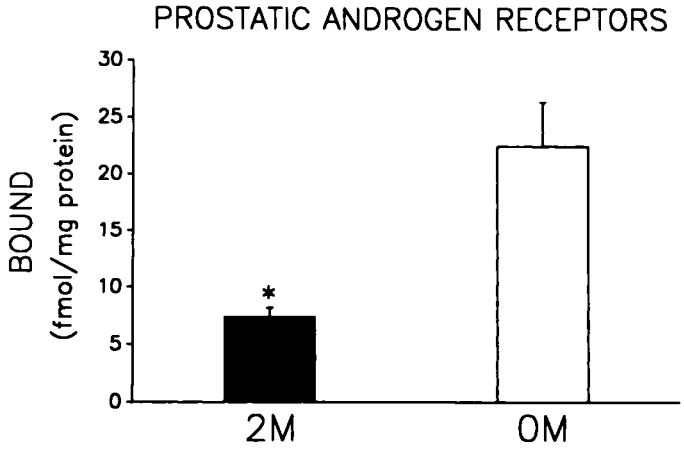

FIG. 3. Androgen binding by high-salt extracts (occupied receptor) of 0M and 2M mouse prostates. Extracts from individual prostates were incubated in duplicate overnight at 4°C with 20 nM [^{3}H]DHT, and bound and free hormone were separated by use of HAP (* $p < 0.01$; n = 7 0M and 2M prostates).

higher ($p < 0.01$) in 0M males than in 2M males (Fig. 3). The values in 0M males ranged from 10.9 to 35.6 fmol/mg protein (22.5 ± 3.9, mean ± SE; n = 7); in 2M males they ranged from 4.0 to 9.3 fmol/mg protein (7.4 ± 0.8, mean ± SE; n = 7).

DISCUSSION

The integrity of the accessory sex organs and their secretions is an important factor in the reproductive success of mice [18]. Either neonatal or prepubertal administration of androgens or estrogens is known to drastically alter the normal development (i.e., morphogenesis and differentiation) and function of the seminal vesicles and prostate [19–21]. Since 0M and 2M male mouse fetuses differ in their circulating levels of T and estradiol, it was of interest to evaluate the developmental consequences (i.e., effects of intrauterine position) on the male accessory sex tissues in adulthood.

Measuring the activities of testicular steroidogenic enzymes, namely Δ^5-3β-hydroxysteroid dehydrogenase/isomerase, 17α-hydroxylase, and $C_{17,20}$-lyase, provided an index of the relative abilities of testes from 0M and 2M males to produce androgens. Although adult levels of circulating T and estradiol have not been reported in 0M and 2M mice, our findings suggest that the observed levels of steroidogenic enzymes alone are probably not responsible for the differences seen in accessory sex organs of 0M and 2M male mice, especially since the mean activities of all three enzymes between the two groups were not significantly different (Table 1).

Prior intrauterine fetal position had dramatic effects on both seminal vesicle and prostate weights in adult mice (Table 2). The increase in 5α-reductase enzyme activity observed in 2M animals was consistent with the significantly heavier ($p < 0.05$) seminal vesicles in these animals compared to those in 0M mice. Adult 2M male mice, either castrated or intact, that had received a silastic capsule implant containing T had significantly greater 5α-reductase in seminal vesicles than did 0M males that had received the same treatment (our unpublished observations). Differences between adult 0M and 2M mice in seminal vesicle weight may have been due to induction of greater concentrations of 5α-reductase by elevated concentrations of T in 2M male fetuses, rather than to differences in circulating T in adulthood.

In contrast, 0M prostates were heavier although the 2M prostates had higher 5α-reductase levels (Table 2). Since androgens and estrogens are important for prostatic growth, both hormone receptors were measured to further investigate the basis of these differences in organ weight. Estrogen receptors reside in both the stromal and epithelial compartments of the prostate in rats [22, 23], but are probably more concentrated in the stroma. It is possible that differences in stroma might account for the differences in prostate weight between 0M and 2M males. Since fetal 0M mouse prostates are exposed to higher levels of circulating estradiol [5], heavier prostates in 0M males could be due to stromal growth induced by estrogen [22]. We had thus predicted that prostates in 0M males would show higher estrogen receptor concentrations than organs in 2M males. We failed to observe any effect of prior intrauterine position on prostatic estrogen binding in 0M and 2M male mice (data not shown).

However, 0M prostates had significantly higher ($p < 0.01$) androgen receptor concentrations (occupied receptor) compared to 2M prostates (Fig. 3), and this could sufficiently increase androgen responsiveness/sensitivity to account for the observation of larger prostates in 0M adults. Whether this striking difference was the result of greater estrogen exposure and/or lesser androgen exposure in the fetus is unclear. The organization of 0M prostates by estradiol may act through stromal instruction and permissive effects in prostatic development, thus leading to the observation of elevation in androgen receptors in our studies [24]. On the other hand, fetal androgens may also play a role in "programming" prostate androgen responsiveness, perhaps through relative down-regulation of androgen receptors in 2M mice, which have higher serum concentrations of T than do 0M males during fetal life.

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