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Androgen Receptors in Brain and Pituitary of Female Rats: Cyclic Changes and Comparisons with the Male[1]

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ABSTRACT

The in vitro binding of a synthetic androgen, methyltrienolone ([^{3}H]-R1881), to brain and pituitary (PIT) cytosol and nuclear extracts was determined in male and female rats. Purified cytosol was prepared from PIT or hypothalamic-preoptic area-amygdala (HPA) and incubated in the presence of 0.1 to 10 nM [^{3}H]-R1881. Scatchard analysis revealed the presence of a single, saturable, high-affinity binding site in PIT cytosol with a dissociation constant (K_d) of 0.42 × 10^{-10} M in females and 0.95 × 10^{-10} M in intact males. The K_d of HPA cytosol was much less in castrated males [0.47 ± 0.05 (SEM) × 10^{-10} M, n=7] and females (0.63 ± 0.1 × 10^{-10} M, n=4) than in intact males (5.8 ± 1.1 × 10^{-10} M, n=8). Treatment of castrated males with dihydrotestosterone (DHT) for 24 h (250 μg/100 g of body weight) increased the K_d of HPA cytosol only slightly (1.6 × 10^{-10} M, mean of two replicates).

Scatchard analysis of salt-extracted nuclear androgen receptor (AR_n) showed a single, high-affinity binding site with similar K_d values in PIT and HPA of intact and castrated, DHT-treated male rats (PIT K_d = 7.3 × 10^{-10} M, 9.3 × 10^{-10} M; HPA K_d = 1.5 × 10^{-9} M, 1.3 × 10^{-9} M, respectively). Competition studies involving a range of several radioinert steroids revealed that the binding of [^{3}H]-R1881 to cytosol (AR_c) and nuclear extract was specific for androgen receptor when triamcinolone acetonide (10 μM) was added. The AR_c and AR_n levels were quantified in PIT, preoptic area (POA), hypothalamus (HT), amygdala, hippocampus, and cortex by single point estimation. Significantly ($p<0.01$) greater amounts of AR_c were detected in PIT of ovariectomized females (32.7 ± 2.9 fmol/mg of protein) than in that of orchidectomized males (22.33 ± 1.6 fmol/mg of protein). The highest levels in the brain were seen in HT and POA. Pituitary AR_c in females varied throughout the estrous cycle. Significantly ($p<0.01$) greater amounts were detected on estrus (45.8 ± 2.2 fmol/mg of protein) and proestrus (39.0 ± 1.9 fmol/mg of protein) than on diestrus (29.2 ± 1.5 fmol/mg of protein). These data confirm the existence of specific receptors for androgen in male and female brain and PIT, and suggest an important role for androgen in the control of PIT hormone secretion in the female.

INTRODUCTION

It is well documented that specific, high-affinity receptors for androgen exist in the brain and pituitary of rodents (Jovan et al., 1973; Barley et al., 1975; Lieberburg et al., 1977; Hannouche et al., 1978). These receptors are localized in regions thought to regulate male reproductive behavior and neuroendocrine function (Davidson and Sawyer, 1961; Christensen and Clemens, 1974; Sar and Stumpf, 1975, 1977). Present theories suggest that steroids act by binding a specific cytosolic receptor and then the receptor is transformed and translocated into the nucleus to initiate a cellular response (Gorski et al., 1968; Jensen, 1982).

Although the aromatization of testosterone to estrogen has been shown to play an important role in

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the biologic action of androgen (Naftolin et al., 1975; Larsson, 1979), there is ample evidence that androgen alone participates in the initiation of male behaviors (Baum, 1979; Beatty, 1979; Soderstein and Gustafsson, 1980) and in the neuroendocrine regulation of pituitary hormone secretion (Swerdloff et al., 1972; Krey et al., 1982). The role of androgen in the female is not well understood. Serum androgen titers vary throughout the estrous cycle and reach peak levels on proestrus (Gay and Tomacari, 1974). These high titers of androgen may regulate the selective release of follicle-stimulating hormone (FSH) on the morning of estrus (Gay and Tomacari, 1974). The administration of dihydrotestosterone, a nonaromatizable androgen, results in an immediate decline in pituitary luteinizing hormone (LH) secretion similar to that seen in males (Swerdloff et al., 1972; Kraulis et al., 1978; Krey et al., 1982). Additionally, exogenous androgen induces male behaviors in female rats. However, the same amount of androgen is less effective in the female than in the male (for review, see Goy and McEwen, 1980).

These studies suggest that androgen helps regulate gonadotropin secretion throughout the estrous cycle of the female rat and that there may be a possible sex difference in the ability of androgen to bind to brain and pituitary tissues. To investigate these two possibilities, we characterized and compared the binding of a synthetic androgen, methyltrienolone (R1881), in brain and pituitary of the male rat to that of the female rat. We first adapted and validated an androgen receptor assay previously described by Hicks and Walsh (1979) and McGinnis et al. (1983) for the rapid and simultaneous estimation of cytosolic and nuclear androgen receptors in small brain regions.

MATERIALS AND METHODS

Animal and Tissue Preparation

Male and female Sprague-Dawley rats (260–300 g) were purchased from Simonsen Breeders (Gilroy, CA) and maintained on a 14L:10D schedule. Food and water were available ad libitum. Castration, when applicable, was performed 4 days prior to use. Tritiated methyltrienolone ([^{3}H]-R1881; New England Nuclear, Boston, MA; 85–90 Ci/mmol) was periodically purified to reduce nonspecific binding on Eastman Kodak silica gel chromagram sheets in the solvent system benzene/ethyl acetate (6:4), and was stored in distilled ethanol at −20°C. The dihydrotestosterone (DHT) for injection was diluted in sesame oil (5 mg/ml) and injected 24 h prior to killing. All other steroids were diluted in distilled ethanol and stored at 2°C until used.

On the day of assay, intact and castrated male and female rats were decapitated (0900–1200 h). The brain and pituitary were removed rapidly from the skull and cooled by placing in crushed ice. Trunk blood was collected and subsequently assayed for steroid hormones. Brain dissections were performed as described by Luine et al. (1974). Whole brains were placed ventral side up on a cold wax plate, and a cut was made with a razor blade just anterior and just posterior to the optic chiasm. With the anterior commissure and optic chiasm as landmarks, a block of tissue was removed from this section. This piece of tissue was designated the preoptic area (POA), although it also contained parts of the anterior hypothalamus, stria terminalis, and diagonal band of Broca. A third slice was made just anterior to the mammillary bodies. With the hypothalamic sulci as lateral boundaries and the top of the third ventricle as the dorsal boundary, the hypothalamus was removed from this slice as a block of tissue. The amydala was obtained by removal of a bilateral triangle of tissue just lateral to the hypothalamic sulci. The cortex was removed as a block of tissue on the dorsolateral edge of this section, and the hippocampus was dissected from the remaining tissue just posterior to the section containing the hypothalamus and amygdala.

Cytosol preparation. Immediately after dissection, all tissues were placed into cold Dounce tissue grinders (Wheaton Scientific, Milville, NJ) and homogenized in either 1 ml (for validation studies) or 200 μl (for single assay) of TEMGD buffer (10 mM Tris, 1.5 mM EDTA, 25 mM molybdate, 10% [v/v] glycerol, 1 mM dithiothreitol; pH 7.4) using 15–20 strokes of a glass pestle with a clearance of 0.003–0.008 cm. The homogenate was transferred to 5 × 20-mm polyethylene centrifuge tubes, and the homogenizer and pesticle were rinsed with 100 μl of TEMGD. This wash was combined with the original homogenate. The entire homogenate was centrifuged at 1000 × *g* for 10 min in a Beckman J-6B centrifuge (Beckman Instr., Palo Alto, CA). The purified cytosol was prepared from the resultant supernatant by recentrifugation at 106,800 × *g* for 10 min in a Beckman Airfuge operated in a cold room at 4°C. Ten microliters of the purified cytosol were used for determination of soluble protein by the method of Lowry et al.

(1951); the remaining cytosol was used for determination of cytosolic androgen receptor (AR_c).

Nuclei preparation. The tissue pellet obtained after the 1000 × *g* centrifugation was washed once with Buffer A (1 mM KH_2PO_4, 0.32 M sucrose, 3 mM $MgCl_2$, 10% glycerol [v/v], 1 mM dithiothreitol; pH 6.8), centrifuged at 1000 × *g* for 10 min, and resuspended in 15 μl of Buffer A containing 3 mg of Cellex 410 (Bio-Rad Labs., Richmond, CA). The Cellex 410 was first washed in distilled ethanol to remove residual pyridines, distilled water (twice), and Buffer A.

One hundred twenty-five microliters of Buffer B (1 mM KH_2PO_4, 2.4 M sucrose, 1 mM $MgCl_2$, 10% glycerol [v/v], 1 mM dithiothreitol; pH 6.8) were carefully added to the suspension and mixed. A purified nuclear preparation was obtained by centrifugation at 57,750 × *g* for 10 min in a Beckman Airfuge (4°C). After centrifugation, the pellicle and sucrose-containing buffers were carefully removed and the walls of the tube were dried with a cotton swab. The remaining pellet, visible with the aid of the Cellex 410, consisted of cellulose fibers and cell nuclei when examined microscopically.

Nuclear androgen receptor (AR_n) was extracted from the purified nuclear pellet with 0.8 M KCl as follows. For each 5 × 20-mm tube, the nuclear pellet was suspended in 52.5 μl of TEBD buffer (10 mM Tris, 1.5 mM EDTA, 0.5 mM bacitracin, and 1 mM dithriothreitol; pH 7.4). After 5 min, an equal volume of TEBDK buffer (TEBD plus 1.6 M KCl; pH 7.4) was added to bring the final salt concentration to 0.8 M. The tube contents were "vortexed" frequently for an additional 25 min. Salt-extracted receptor was separated from Cellex and nuclei by centrifugation (37,000 × *g* for 5 min) and the salt extraction procedure was repeated to maximize receptor yield. Supernates from both salt extractions were combined and used in the exchange assay for AR_n. Pelleted material was assayed for DNA by the method of Burton (1956), by means of the Giles and Meyers modification (1965), in a total volume to 1.025 ml.

Owing to the small path length required when 5 × 20-mm centrifuge tubes are used, our preliminary studies showed that a 10-min centrifugation at 106,000 × *g* is adequate for the purification of cytosol, and 10 min at 57,750 × *g* is adequate to pellet nuclei through 2 M sucrose buffer. Cellex 410 was added during the preparation of the nuclear pellet because of the small amount of tissue retained after fractionation. Cellex 410 had previously been used to immobilize DNA to cellulose for DNA-affinity chromatography (Moss et al., 1981). In our assay, Cellex 410 was used primarily as a marker for the nuclear pellet derived from the 57,750 × *g* spin. It was further noted that the addition of Cellex resulted in a 50% increase in pelleted DNA.

Androgen Binding Kinetics

Pituitary or hypothalamus-preoptic area-amygdala (HPA) of 4–6 rats was pooled for kinetics studies. The groups studied were: 1) intact males; 2) castrated males; 3) castrated males given DHT (250 μg/100 g of body weight) 24 h prior to death; 4) intact females, killed at random stages of the estrous cycle; and 5) ovariectomized females. Pituitary or HPA tissues were homogenized in 1 ml of TEMGD. Homogenizers were rinsed with 500 μl of TEMGD. The homogenate was divided equally between six 5 × 20-mm centrifuge tubes for processing. The resulting purified cytosol preparation or nuclear extract was combined before being portioned (100 μl) into 1.5-ml conical incubation tubes containing [^{3}H]-R1881 (0.1 nM to 10 nM) in 50 μl of TEMGD or TEBD. A parallel set of incubation tubes containing an additional 100-fold excess of cold R1881 was carried to determine nonspecific binding. Triamcinolone acetonide (10 μM) was added to all tubes to prevent binding of R1881 to the progesterone or corticosterone receptor.

The [^{3}H]-R1881 and cytosol were incubated for 40–45 h at 0–4°C. Then the bound [^{3}H]-R1881 was separated from free [^{3}H]-R1881 on miniature Sephadex LH-20 columns made from 1-ml polyethylene pipette tips plugged with glass fiber. The LH-20 was swollen overnight in TEMGD (AR_c assay) or TEBD (AR_n assay), packed to a bed height of 5.5 cm, and equilibrated with at least 600 μl of TEMGD or TEBD/TEBDK (1:1) immediately prior to use. One hundred twenty-five microliters of incubate were layered onto the top of the bed and washed into the bed with 100 μl of the appropriate buffer. Flow was allowed to stop for 30 min; then columns were eluted into minivials with 600 μl of the appropriate buffer. Three milliliters of Atomlite (New England Nuclear) were added and radioactivity was counted in a Packard Tricarb 460 scintillation counter to less than 5% error at 46% efficiency. Counts were automatically converted to disintegrations per minute and corrected for internal quenching. Specific binding was

determined by subtraction of nonspecific binding from total counts bound. The [^{3}H]-R1881 bound was expressed as femtomoles per milligram of protein or DNA in all cases, and the data were analyzed according to the method of Scatchard (1949).

To determine the effect of time on [^{3}H]-R1881 binding, we incubated the cytosol with the ligand at a final concentration of 4 nM for AR_c and 6 nM for AR_n at time points ranging from 4 h to 72 h. To determine the linearity of the cytosol protein concentration, we incubated 4 nM [^{3}H]-R1881 with cytosol in a range of 25–500 μg of protein per incubation tube. To determine the optimum salt concentration for extraction of AR_n, we utilized a final KCl concentration from 0.2 M to 0.8 M.

Binding Specificity

The specificity of [^{3}H]-R1881 binding to cytosol receptor was determined in pituitary and HPA from intact and castrated males and females. Specificity of [^{3}H]-R1881 binding for AR_n was determined in pituitary and HPA of intact males and castrated, DHT-treated males. Purified cytosol and nuclear extract were prepared as previously described, and incubation in the presence of 4 nM (AR_c) or 6 nM (AR_n) [^{3}H]-R1881 for 40–45 h at 0–4°C. Radioinert steroids (DHT, testosterone, estradiol-17β, progesterone, or corticosterone) were added to both total and nonspecific binding incubation tubes at a 10-, 50-, or 100-fold excess in relation to [^{3}H]-R1881. Free and bound [^{3}H]-R1881 were separated on LH-20 minicolumns. Specific binding was calculated as a percentage of the specific binding in incubation tubes containing no competing steroid.

Single point assay. Using the binding parameters determined in the previously described experiments, we developed a single point assay for the simultaneous determination of nuclear and cytosolic androgen receptors in small quantities of tissue. Pituitary and brains were removed and processed as previously described. Brain tissue from a single region from individual rats was homogenized in 200 μl of TEMGD, and homogenizers were rinsed with 100 μl of TEMGD. After centrifugation at 106,800 × *g*, the resulting purified cytosol preparation was diluted to a 220-μl total volume with TEMGD when necessary. The protein content was determined in a 10-μl aliquot. A 100-μl aliquot was added to a 1.5-ml conical incubation tube containing 50 μl of TEMGD with 4 nM [^{3}H]-R1881, and 10 μm triamcinolone acetonide, to determine total binding. Nonspecific binding was determined by a paired incubation with 800 nM cold R1881 in addition to [^{3}H]-R1881 and triamcinolone acetonide.

Two extractions of the purified nuclear pellet with TEBD and TEBDK resulted in approximately 210 μl of nuclear extract containing AR_n. One hundred-microliter aliquots were added to total and nonspecific binding tubes containing 6 nM [^{3}H]-R1881 in 50 μl of TEBD/TEBDK (1:1). The concentrations of triamcinolone acetonide and radioinert R1881 were the same as in the binding assay for AR_c. All incubations lasted 40–45 h at 0–4°C.

Free and bound [^{3}H]-R1881 were separated on Sephadex LH-20 columns as previously described. Nonspecific binding ranged from 30 dpm in the preoptic area to 125 dpm in the pituitary for both AR_c and AR_n. This represented from 8% to 65% of the total bound counts, the percentage depending on tissue and treatment group.

Steroid Radioimmunoassay

Serum steroid titers were determined by radioimmunoassay. Steroids were extracted from sera with ether and separated by Sephadex LH-20 column chromatography with a hexane/benzene/methanol (65:20:15) system. Specificity and reliability of these methods have been described elsewhere (Resko et al., 1980).

Statistics

The data obtained from single point determinations were analyzed by one-way analysis of variance. Each brain region was analyzed independently. Post hoc comparison of treatment differences was accomplished with the Neuman-Keuls test (Winer, 1977).

RESULTS

In order to first validate our methods, our initial experiments were performed on brain and pituitary tissues pooled from several animals. Subsequently, we developed a single point assay for the determination of androgen receptor in tissue from individual animals. Saturation studies, involving a range of [^{3}H]-R1881 concentrations from 0.1 to 10 nM, revealed the presence of a single saturable, high-affinity binding site in both brain and pituitary tissue. In Fig. 1A, Scatchard analysis of data obtained from pituitary cytosol of intact and castrated male (0.9×10^{-10} M

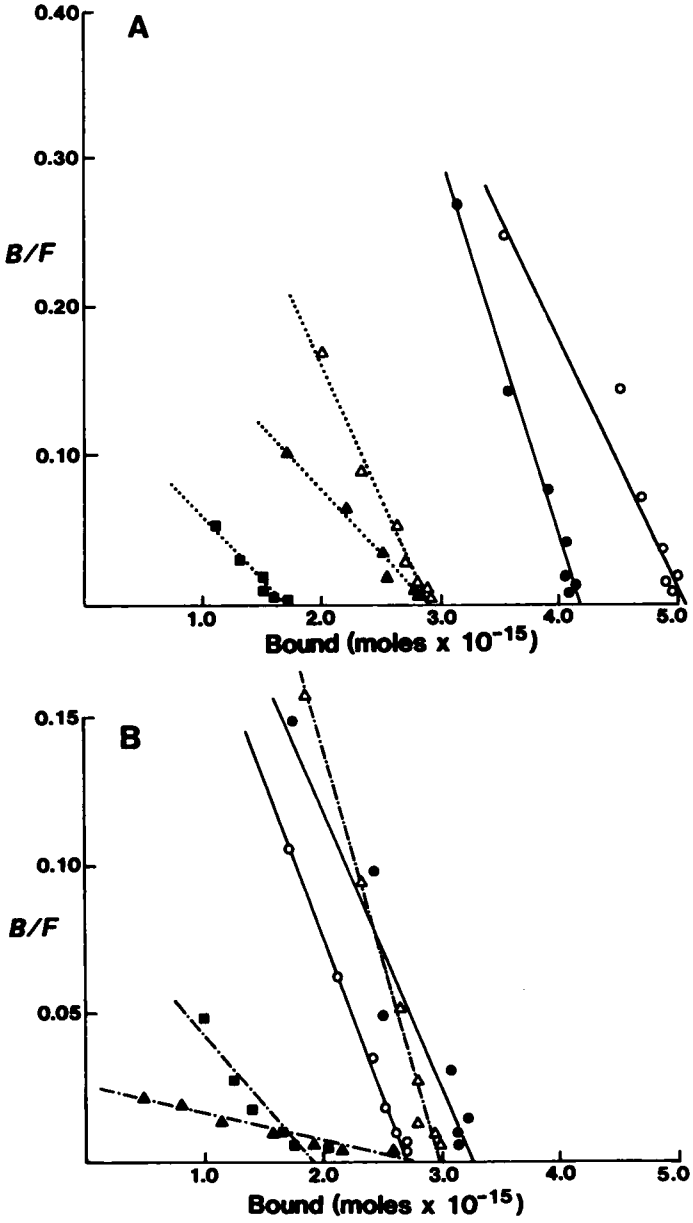

FIG. 1. Scatchard analysis of [^{3}H]-R1881 binding to rat pituitary (*A*) and hypothalamus-preoptic area-amygdala (*B*) cytosol of intact males (▲) and females (●), gonadectomized males (△) and females (○), and castrated males given DHT (250 mg/kg of body weight; ■). Gonadectomized rats were killed 4 days after surgery. Intact females were killed at random stages of the estrous cycle. Cytosols from 5 or 6 rats were pooled and incubated with 0.1 to 10 nM [^{3}H]-R1881 with and without a 200-fold excess of cold R1881. Units of measurement were moles × 10^{-15} per mg protein.

and 0.3 × 10^{-10} M, respectively; mean of two replicates) and intact and castrated female (0.4 × 10^{-10} M and 0.3 × 10^{-10} M, respectively; mean of two replicates) rats and castrated, DHT-treated males (1.06 × 10^{-10} M, mean of two replicates) suggests similar binding affinities for [^{3}H]-R1881 between the groups. As expected, the number of binding sites as estimated by Scatchard analysis increased after castration of male rats from 11.5 × 10^{-15} mol/mg of protein to 20.2 × 10^{-15} mol/mg of protein and decreased after DHT treatment (7.0 × 10^{-15} mol/mg of protein). Pituitary AR_c was greater in intact (32.0 × 10^{-15} mol/mg of protein) and castrated (31.5 × 10^{-15} mol/mg of protein) females than in castrated males. In these initial studies, there did not appear to be a difference between intact females selected at random stages of the estrous cycle and castrated females.

In HPA cytosol (Fig. 1B), Scatchard analysis of [^{3}H]-R1881 binding showed an increase in the affinity of the AR_c after castration of the male [dissociation constant (K_d) = 0.47 ± 0.05 × 10^{-10} M, n=7]. This was represented by a 10-fold decrease in K_d, and was similar to the K_d found in intact (0.65 ± 0.12 × 10^{-10} M, n=4) and castrated (0.63 ± 0.10 × 10^{-10} M, n=4) female HPA cytosol. Treatment of the castrated male with DHT for 24 h did alter the K_d (1.6 × 10^{-10} M, n=2) slightly, but did not return it to the level of the intact male (K_d = 5.81 ± 1.10 × 10^{-10} M, n=8). No differences in HPA cytosol B_{max} were seen between castrated males and females (8.9 ± 0.9 × 10^{-15} mol/mg of protein and 11.8 ± 2.1 × 10^{-15} mol/mg of protein, respectively). However, HPA of intact males and castrated, DHT-treated males (5.8 ± 0.5 × 10^{-15} mol/mg of protein and 3.0 × 10^{-15} mol/mg of protein, respectively) contained less AR_c than HPA of castrated rats, and additionally contained measurable amounts of AR_n (73.9 ± 8.0 × 10^{-15} mol/mg of DNA and 65.2 × 10^{-15} mol/mg of DNA, respectively). In no instance did we detect changes in nonspecific binding.

Nuclear androgen receptor was extracted with 0.8 M KCl. This concentration seemed optimal in our assay conditions. Decreasing the KCl concentration to 0.6 or 0.4 M lowered the amount of extracted AR_n. Extraction with 0.2 M KCl yielded almost no [^{3}H]-R1881 binding. Scatchard analysis of AR_n binding in intact males revealed a single, saturable, high-affinity binding site that was similar in brain (K_d = 1.69 ± 0.32 × 10^{-9} M) and pituitary (Fig. 2, K_d = 0.733 × 10^{-9} M).

The length of incubation was determined by incubation of cytosol and nuclear extract with 4–6 nM [^{3}H]-R1881 for various times ranging from 4 to 72 h. Binding of AR_c and AR_n in pituitary and HPA was at equilibrium within 30 h in all cases. For this reason,

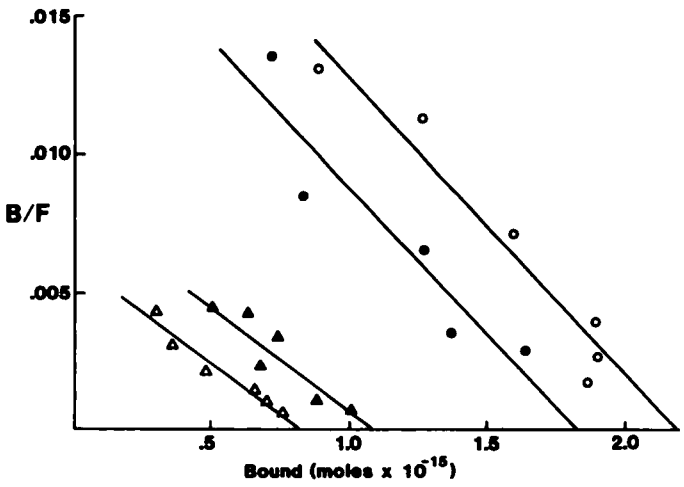

FIG. 2. Scatchard analysis of [^{3}H]-R1881 binding to nuclear extract of pituitary (●, ○) and hypothalamus-preoptic area-amygdala (HPA; ▲, △) of intact male (*closed symbols*) and castrated, DHT-treated male (250 mg/kg of body weight, killed 24 h later) (*open symbols*) rats. Receptor was extracted with 0.8 M KCl. The nuclear extracts of 5 or 6 rats were pooled and incubated with 0.1 to 10 nM [^{3}H]-R1881 in the presence and absence of a 200-fold excess of cold R1881. The K_d = 0.61 × 10^{-9} M and 0.64 × 10^{-9} M for intact and DHT-treated pituitary, respectively, and 0.84 × 10^{-9} M and 0.91 × 10^{-9} M for intact and DHT-treated HPA, respectively. Units of measurements were moles × 10^{-15} per mg DNA.

all subsequent incubations lasted 40–45 h. In all instances, binding was stable at 0–4°C for at least 72 h. Additional studies also showed that this binding was linear in a range of protein concentrations from 25 to 500 μg per incubation tube.

Binding Specificity

To test the specificity of [^{3}H]-R1881 binding under our in vitro assay conditions, we incubated pituitary and HPA cytosol and nuclear extract with [^{3}H]-R1881 and a variety of radioinert steroids (Fig. 3). In the presence of 10- to 100-fold molar excess of progesterone and corticosterone, the binding of [^{3}H]-R1881 was decreased only slightly. In all cases, estradiol-17β was a much better competitor in our system than progesterone or corticosterone. However, it was still less than 10% as effective as testosterone or DHT in competing with [^{3}H]-R1881. The decreased [^{3}H]-R1881 binding after progesterone, corticosterone, and estradiol may represent binding of these steroids to androgen receptor rather than the binding of [^{3}H]-R1881 to progesterone, corticosterone, and estrogen receptor. Both testosterone and DHT were excellent competitors with R1881 for androgen receptor, but the addition of DHT consistently resulted in greater decreases in [^{3}H]-R1881 binding.

Single Point Studies

Our initial studies on AR_c levels in several brain areas of the male rat demonstrated the distribution and physiologic dynamics of the androgen receptor. As seen in Fig. 4, differences existed in the various brain regions in regard to the quantity of AR_c. Highest levels were found in hypothalamus, and lowest levels in cortex. Pituitary levels were consistently higher than those found in any brain region. Castration of the male significantly increased AR_c in pituitary and all brain regions ($p<0.01$) with the exception of the amygdala and the cortex, in which

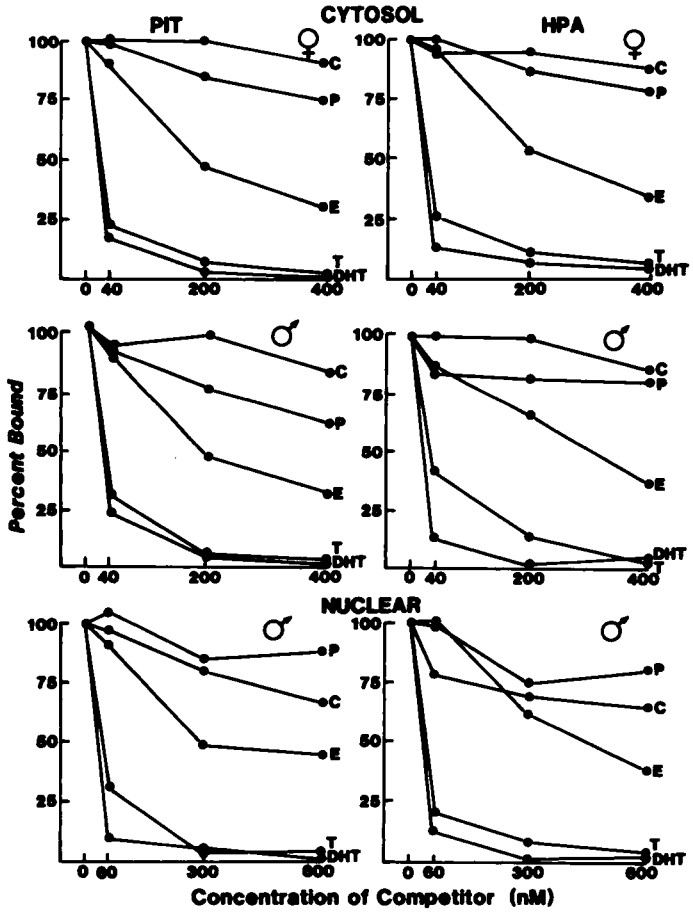

FIG. 3. Competition of various radioinert steroids with the binding of [^{3}H]-R1881 to pituitary and hypothalamus-preoptic area-amygdala cytosol and nuclear extract. The [^{3}H]-R1881 was used at a concentration of 4 nM in cytosol assays and 6 nM in nuclear assays. Binding is expressed as a percentage of that obtained in the presence and absence of cold R1881 only. *P* = progesterone; *C* = corticosterone; *E* = estradiol-17β; *T* = testosterone; *DHT* = dihydrotestosterone. Units of measurement were moles × 10^{-15} per mg protein (cytosol) or DNA (nuclear).

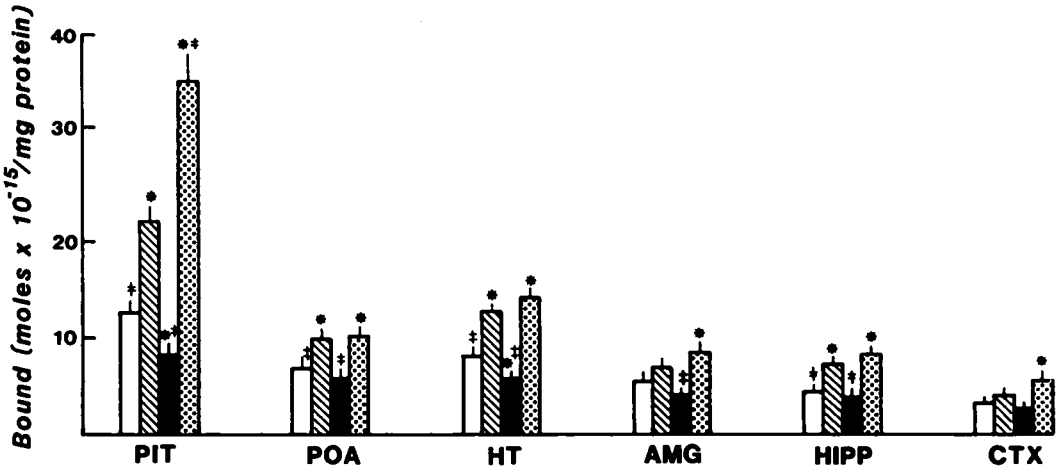

FIG. 4. Cytosolic androgen receptors in the pituitary (*PIT*) and various brain regions of intact males (*open bars*, n=10), castrated males (*striped bars*, n=7), castrated males given dihydrotestosterone (250 mg/kg BW; *solid bars*, n=8), and ovariectomized females (*dotted bars*, n=7). Castration and ovariectomy were performed 4 days prior to death of the rats. Dihydrotestosterone was administered 24 h prior to death. Vertical lines represent the standard errors of the mean. *POA* = preoptic area; *HT* = hypothalamus; *AMG* = amygdala; *HIPP* = hippocampus; *CTX* = cortex. The * represents groups that are significantly different ($p<0.05$) from intact males. The † represents groups that are significantly different ($p<0.05$) from castrated males.

increases after castration did not reach significance. Twenty-four hours after the administration of DHT to castrated males, AR_c was at or below the level found in the intact male.

The simultaneous determination of AR_n in intact, castrated and castrated, DHT-treated males showed high levels in intact males, and these disappeared after castration (Table 1). The administration of DHT to castrated males increased plasma androgen to supraphysiologic levels (Table 2), but the amounts of pituitary and HPA AR_n were in turn comparable to those in the intact male (Table 1). Interestingly, AR_c in castrated female pituitary was significantly greater than that in the castrated male pituitary ($p<0.01$). This sex difference was not present in any brain tissue examined. Further evidence that this male-female difference is real was found when total binding sites were estimated by the method of Scatchard. Differences were again seen in pituitary but not brain.

Further studies on AR_c and AR_n levels in female pituitary and brain showed significant changes in pituitary AR_c and AR_n throughout the estrous cycle (Fig. 5, Table 3). The AR_c levels reached a peak on estrus morning, and lowest levels were found on diestrus morning. These changes in AR_c were not present in brain. Pituitary AR_n was significantly greater on proestrus and estrus versus that on

TABLE 1. In vitro nuclear [^{3}H]-R1881 binding in male and female rats.

		Nuclear R1881 binding (fmol/mg DNA)[b]					
	n[a]	PIT	POA	HT	AMG	HIPP	CTX
Intact male	8	156.9 ± 26.0[c]	99.6 ± 26.9	106.8 ± 14.2	75.4 ± 11.4	73.5 ± 45.5	69.9 ± 17.4
Castrated male[d]	7	ND	ND	ND	ND	ND	ND
Castrated male + DHT[e]	8	261.7 ± 44.1	92.6 ± 14.4	105.2 ± 18.7	53.2 ± 12.5	50.5 ± 8.3	38.1 ± 4.2
Ovariectomized female	7	ND	ND	ND	ND	ND	ND

[a] n = number of animals studied in each group.

[b] PIT = pituitary; POA = preoptic area; HT = hypothalamus; AMG = amygdala; HIPP = hippocampus; CTX = cortex; ND = not detectable.

[c] Data presented as means ± SEM.

[d] Animals were castrated 4 days before killing.

[e] Animals were castrated 4 days and received 250 mg/kg body weight DHT 24 h before killing.

TABLE 2. Serum steroid concentrations in male and female rats.[a]

	n	Estradiol (pg/ml)	DHT (pg/ml)	Testosterone (pg/ml)
Intact male	6	2.10 ± 0.5	869.0 ± 0.257	2676.0 ± 188
Castrated male	5	2.30 ± 0.7	3.2 ± 1.1	12.0 ± 1.8
Castrated male + DHT	8	3.30 ± 0.3	6000.0 ± 1528	59.0 ± 7.7
Ovariectomized female	7	2.96 ± 0.3	21.1 ± 2.3	23.7 ± 4.7

[a]See Table 1 for abbreviations and explanation of treatments.

diestrus, but was consistently low and near the limits of detectability of our assay. The HPA AR_n levels were nondetectable in the female.

Plasma steroid values are shown in Table 3. Estradiol levels were significantly elevated on proestrus and estrus ($p<0.001$). The DHT titers were highest on estrus, and serum testosterone was significantly elevated on proestrus ($p<0.01$).

DISCUSSION

We have characterized and compared the cytosolic and nuclear androgen receptors in brain and pituitary tissues of male and female rats. Additionally, we have quantified androgen receptor in several brain regions. The results suggest a role for androgen in pituitary and perhaps brain function in the female rat.

In several earlier studies, the presence of a high-affinity receptor for androgen in both pituitary and brain tissue was observed (Jovan et al., 1973; Barley et al., 1975; Lieberburg et al., 1977; Hannouche et al., 1978). In general, our observations of differing dissociation constants between AR_c of castrated and intact male HPA suggest that the brain is more sensitive to androgen in the absence of endogenous androgen. Although the lower K_d that we report for intact male HPA may be explained by endogenous androgen competing for the receptor, several observations appear to rule out this possibility. Alterations in the K_d of pituitary AR_c do not differ in the same fashion as those observed in HPA; therefore, the effect may be specific for brain tissue. The administration of DHT to castrated male rats did increase the K_d of HPA and pituitary AR_c; however, only in pituitary was this increase in K_d comparable to that seen in the intact male. The DHT did not increase the K_ds of HPA to those of the intact male, even though subsequent quantification of AR_n resulted in values that

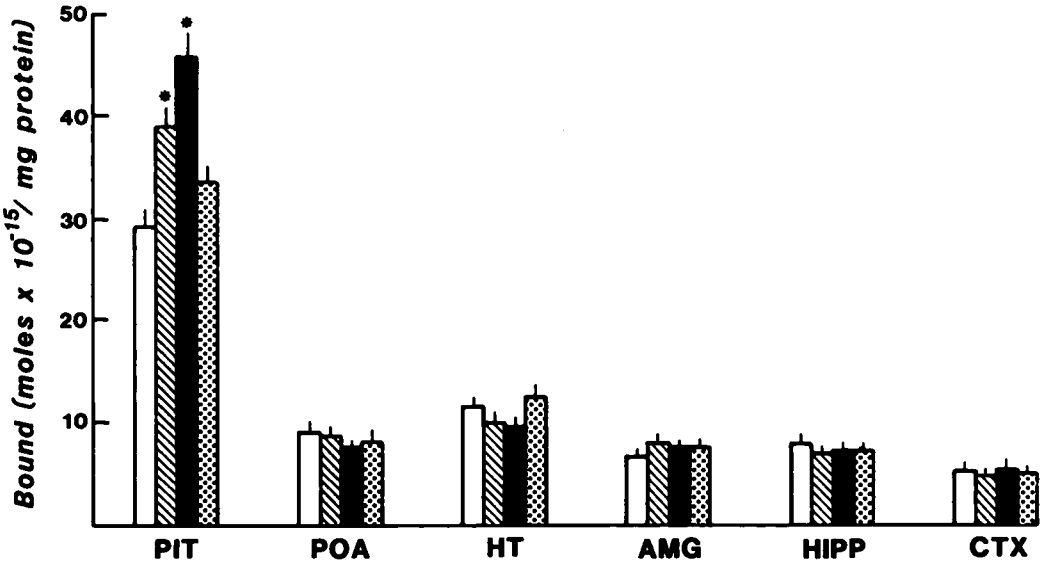

FIG. 5. Changes in cytosolic androgen receptor in the pituitary (*PIT*) and various brain regions of the female rat throughout the estrous cycle. Animals were killed between 0900 and 1100 h on diestrus (*open bars*, $n=5$), proestrus (*striped bars*, $n=8$), estrus (*solid bars*, $n=8$), or metestrus (*dotted bars*, $n=5$). Vertical bars represent the standard errors of the mean. *POA* = preoptic area; *HT* = hypothalamus; *AMG* = amygdala; *HIPP* = hippocampus; *CTX* = cortex. The * indicates a significant ($p<0.01$) difference from the diestrus value.

TABLE 3. Nuclear androgen receptor in anterior pituitary and serum steroid titers throughout the estrous cycle of the female rat.

	n	Nuclear R1881 binding (fmol/mg DNA)	Plasma steroids		
			Estradiol	DHT (pg/ml plasma)	Testosterone
Diestrus	5	4.9 ± 1.6	7.2 ± 0.8	267.0 ± 76	136.6 ± 14.5
Proestrus	8	10.9 ± 1.4*	48.5 ± 8.2*	262.8 ± 51.4	413.4 ± 69.2*
Estrus	8	10.4 ± 2.3*	24.2 ± 6.4*	478.6 ± 255.5	176.8 ± 30.5
Metestrus	4	7.1 ± 0.9	4.8 ± 0.9	216.6 ± 44.4	98.5 ± 15.6

*$p<0.05$ compared to diestrus.

were equal to or greater than that of the intact male. Additional studies in this laboratory, involving ammonium sulfate precipitation of the intact and castrated male HPA AR_c to separate endogenous steroid from receptor protein, have shown no effect on the K_d (Handa, unpublished observations).

These data may indicate the presence of a testicular inhibitor that interferes with the binding of androgen to its cytosolic receptor, or alternatively, a substance of neural origin that alters the affinity of the HPA AR_c. However, if this latter substance is androgen regulated, it requires at least 24 h to be expressed since DHT treatment does not completely return the HPA cytosol K_d to that of the intact male.

We have also shown that the binding of [^{3}H]-R1881 is specific for androgen receptor. The administration of triamcinolone acetonide to the incubation tube prevents the nonspecific binding of [^{3}H]-R1881 to the progestin and corticosterone receptors (Hicks and Walsh, 1979). As our competition studies show, progesterone and corticosterone are both weak competitors for [^{3}H]-R1881. The small decrease in [^{3}H]-R1881 binding after the addition of cold estradiol-17β, progesterone, and corticosterone may reflect the binding of cold steroid to the androgen receptor. As previously reported, all of these steroids possess some affinity for the androgen receptor (Sheridan, 1983).

Utilizing the biochemical parameters of [^{3}H]-R1881 binding described in our initial studies involving pooled pituitary and HPA, we have validated a single point assay for the simultaneous determination of AR_c and AR_n in small tissue samples. This is a modification of the existing androgen binding assays of Hicks and Walsh (1979) and McGinnis et al. (1983). We have also utilized the Beckman Airfuge to measure AR_n in small volumes by adapting the existing exchange assays for androgen receptor (McGinnis, 1983) and estrogen receptor (Roy and McEwen, 1977). The use of the Airfuge also decreases the time of centrifugation and thus makes this assay extremely rapid.

We have established the biologic validity of this assay by measuring AR_c and AR_n in pituitary and several brain regions of intact males and castrated males given DHT. Our values are in agreement with previous reports on pituitary (Sheridan, 1983), and the distribution throughout the brain appears to agree with previously published distributions arrived at by autoradiographic techniques (Sar and Stumpf, 1975, 1977). Additionally, as previously shown (McGinnis et al., 1983), AR_c rises after castration and decreases following androgen treatment, whereas AR_n is nondetectable after castration and increases following DHT treatment.

In our studies we detected a greater amount of AR_c in the pituitary gland of gonadectomized females than in castrated males. This difference was found when the number of binding sites was estimated by single point analysis and when it was estimated by Scatchard analysis of pooled pituitaries. Additionally, this sex difference was specific for pituitary tissue and was not detected in any brain region. Our observations that more AR_cs are present in the pituitaries of gonadectomized females than in those of males and that pituitary AR_c shows variations throughout the estrous cycle of the female, with highest levels being reached on estrus and lowest levels being attained on diestrus, suggest an important role for androgen in cyclic pituitary hormone secretion.

In previous studies androgen was implicated in the selective release of FSH that occurs on estrus morning (Gay and Tomacari, 1974) because treatment with an antibody to testosterone abolishes it. This rise of FSH is primarily under pituitary regulation, because

it continues after treatments that block luteinizing hormone-releasing hormone stimulation of the pituitary (Blake et al., 1982; Condon et al., 1984). In addition, androgen increases FSH and prolactin release from pituitary cells in vitro (Tang and Spies, 1975; Haug and Gautvik, 1976), and the selective retention of tritiated DHT in gonadotropes has been shown autoradiographically (Dubois et al., 1978) and in purified pituitary cell populations (Thieulant and Duvall, 1985).

Changes in pituitary AR_c throughout the estrous cycle of the female resemble those reported for progesterone receptors (McGinnis et al., 1981); however, the time course is different since progestin receptors peak on proestrus morning and androgen receptors peak on estrus morning. The increases in progestin receptor are a function of rising estrogen titers (McGinnis et al., 1981; Clark et al., 1982), and it is possible that estrogen also controls the cyclic changes in pituitary androgen receptor. A number of earlier studies did, in fact, detect estrogen-induced increases in cytosolic androgen receptor in a variety of other tissues (Tokarz et al., 1978; Moore et al., 1979; Rance and Max, 1984).

Small increases in pituitary AR_n were also detected in the female on proestrus and estrus. It is likely that the level of cytosolic receptor for steroid hormone is much greater than that required to elicit a cellular response. Whether or not these nuclear levels increase further on proestrus evening, in unison with pituitary gonadotropin secretion, remains to be determined. The increases in pituitary AR_c on proestrus and estrus may represent a hypersensitivity of the female pituitary gland to androgen. These studies, then, provide evidence of cellular changes within the pituitary gland that are necessary for androgen action. These changes occur at critical times in the female estrous cycle and may be important to an understanding of the female patterns of gonadotropin secretion.

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