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Estrogen-Induced Sexual Receptivity and Localization of ^{3}H-Estradiol in Brains of Female Mice: Effects of 5α-Reduced Androgens, Progestins and Cyproterone Acetate[1]

WILLIAM G. LUTTGE, THOMAS W. JASPER, HARRY E. GRAY AND CINDY S. SHEETS

Department of Neuroscience and Center for Neurobiological Sciences
University of Florida College of Medicine, Gainesville, FA 32610

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LUTTGE, W. G., T. W. JASPER, H. E. GRAY AND C. S. SHEETS. *Estrogen-induced sexual receptivity and localization of ^{3}H-estradiol in brains of female mice: effects of 5α-reduced androgens, progestins and cyproterone acetate.* PHARMAC. BIOCHEM. BEHAV. **6**(5) 521–528, 1977. – Sexual receptivity induced in ovariectomized CD-1 mice with chronic daily administration of estradiol benzoate (E_2B) was blocked by concurrent administration of the 5α-reduced androgen, dihydrotestosterone (DHT). Receptivity was restored in these females with progesterone-, but not with dihydroprogesterone-priming 6 hr prior to testing. Delaying the DHT injections until 12 hr after the E_2B injections greatly reduced its inhibitory properties. Receptivity in E_2B-primed females was also blocked by concurrent treatment with cyproterone acetate and 3α-, but not 3β-androstanediol. Pretreatment with DHT, or 3α- or 3β-androstanediol failed to consistently affect ^{3}H-estradiol accumulation in crude nuclear and supernatant fractions from brain and pituitary.

Sexual receptivity Dihydrotestosterone Androstanediol Cyproterone acetate
Dihydroprogesterone Progesterone Estrogen ^{3}H-Estradiol Hypothalamus

SEXUAL RECEPTIVITY in ovariectomized mice and other rodents can readily be induced with systemic administration of estradiol benzoate[2] (E_2B) and progesterone. In mice and rats it has been shown that although progesterone clearly facilitates the induction of receptivity [2, 4, 6, 20, 25], it is not an absolute requirement since chronic treatment with E_2B will stimulate receptivity even without progesterone administration [5, 7, 20]. The 5α-reduced progestin, dihydroprogesterone (DHP), can also facilitate receptivity in estrogen-primed rats and CD-1 mice [9, 13, 26]. We have recently shown that the induction of receptivity in CD-1mice is blocked when the 5α-reduced androgen, dihydrotestosterone (DHT), is given concurrently with E_2B at 54 and 30 hr and concurrently with DHP at 6 hr prior to testing. Since an identical E_2B and DHT injection paradigm combined with progesterone rather than DHP priming still induced high levels of receptivity, we concluded that DHT blocked receptivity by specifically inhibiting the actions of DHP [13]. This conclusion also leads to the speculation that DHP and progesterone facilitate the induction of receptivity in estrogen-primed mice by different molecular mechanisms. Baum and Vreeburg [1] independently demonstrated that DHT treatment can also block the induction of sexual receptivity in rats produced by chronic treatment with E_2B and that progesterone administration can overcome most, but not all of this inhibition. Reexamination of our findings in mice in light of these observations on rats suggested that DHT may also be directly inhibiting the behavioral actions of E_2B in mice and that progesterone is better able to overcome this inhibition than is DHP.

In the present series of experiments we have examined this hypothesis by first studying the effects of DHT on estrogen-induced sexual receptivity in ovariectomized CD-1 mice. We then examined the interactive effects of progesterone and DHP on DHT-inhibition of estrogen-induced receptivity. The effects of the 5α-reduced androgens, 3α- and 3β-androstanediol (3α- and 3β-Adiol), and of the anti-androgen, cyproterone acetate (CA), were studied since 3α-Adiol and CA have been shown to inhibit estrogen- or

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[2] All steroid trivial names are defined in the Methods section.

estrogen plus progesterone-induced receptivity in rats [1,17]. As an initial attempt to uncover the mechanism of 5α-reduced androgen inhibition of estrogen-induced sexual receptivity, we examined the effects of these steroids on the regional and subcellular localization of ^{3}H-estradiol in brain and pituitary.

METHOD

Animals

For each study adult albino CD-1 female and male mice were purchased from Charles Rivers Laboratories (Wilmington, MA). All animals were housed individually or in groups of six (Experiment 4 only) with food and water available at all times. The animal colony lights were left on from 2100 to 0900 hr. Based on this time clock all morning injections in the behavioral experiments were given between 0900 and 1200 hr, evening injections were given 12 hr later, while all behavioral tests were scheduled between 0900 and 1200 hr. The injections and decapitations in the isotope study were scheduled between 0830 and 2230 hr. All surgery was performed under methoxyflurane anesthesia. The animal colony of the University of Florida College of Medicine has been accredited by the American Association for Accreditation of Laboratory Animal Care.

Hormones and Anti-Hormones

Estradiol benzoate (1,3,5(10)-estratrien-3,17β-diol 3-benzoate) (E_2B), dihydrotestosterone (5α-androstan-17β-ol-3-one) (DHT), dihydroprogesterone (5α-pregnan-3,20-dione) (DHP), progesterone (4-pregnen-3,20-dione), 3α-androstanediol (5α-androstan-3α,17β-diol) (3α-Adiol), and 3β-androstanediol (5α-androstan-3β,17β-diol) (3β-Adiol) were purchased from Steraloids, Inc. (Wilton, NH). Cyproterone acetate (6-chloro-17-acetoxy-1α,2α-methylene-4,6-pregnadiene-3,20-dione) (CA) was generously provided by Schering A.G. (West Berlin). ^{3}H-Estradiol (6,7-^{3}H-1,3,5(10)-estratrien-3,17β-diol) ^{3}H-E_2 (spec. act., 49.3 Ci/mmole) was purchased from New England Nuclear (Boston, MA).

All unlabeled steroids except E_2B were prepared for injection by either dissolving (DHT, progesterone and DHP) or suspending (3α- and 3β-Adiol) them in a benzyl benzoate-peanut oil solution (20:80, v:v). Uniformly dispersed micro-crystalline suspensions were prepared by sonication in an ultra-sonic water bath. E_2B was dissolved in peanut oil. All unlabeled hormone SC injections in the behavioral experiments were delivered in 0.1 cc volumes, while those in the isotope experiment were given in 0.2 cc. ^{3}H-E_2 was prepared for iv injection by evaporation of the benzene and ethanol solvents under a stream of nitrogen gas at room temperature. The dried residue was dissolved in 20% ethanol and stored at 2° C.

Sexual Behavior Tests

All sexual behavior testing was done in the animal colony under dim red light illumination. Whenever possible testing of individual animals was done without the observer's knowledge of the group from which the animal was derived. Each test was initiated by placing the test female in a 12.5 × 28 × 28 cm testing arena containing a sexually vigorous CD-1 male mouse. Males were permitted to mount the females and the lordosis quotient (L/M × 100) was computed. Tests were terminated after the first 7 mounts if these were all accompanied by a lordosis or nonlordosis response (i.e., L/M = 100 or 0) or after the first 10 mounts if a mixture of lordosis and nonlordosis responses was observed. Only mounts with pelvic thrusting were counted. The female's response to mounting stimulation was scored as a lordosis if her head was level or elevated with respect to the ground and if her back was arched concavely with the perineal region raised and the rear legs extended. This posture typically permitted the male to achieve intromissions. If a given male failed to either initiate or continue mounting, the female was moved to another testing arena containing a different male.

Tissue and Plasma Isolation

For tissue and plasma isolation in Experiment 4, each female was anesthetized with methoxyflurane and its chest cavity opened. Using a heparinized syringe, a sample of cardiac blood was taken, transferred to a heparinized tube, centrifuged at approximately 1000 × g for 10 min and a 50–100 μl sample of plasma obtained. After blood collection each female was perfused with 5–10 ml of ice-cold saline and decapitated. The brain was immediately removed, rinsed in ice-cold homogenization solution and placed on an ice-cold glass plate resting on a tray of ice. The whole pituitary (PIT) was removed from the sella turcica and quickly weighed on a Mettler H-20 analytical balance and transferred to an ice-cold homogenization tube containing 200 μl of the homogenization solution. The brain was dissected on the glass plate as described in our earlier publications [15,16] and each sample pre-cooled and then weighed and transferred to homogenization tubes as described for the PIT samples. The following weights (mg) for brain samples was recorded ($\bar{X} \pm$ SEM): cerebral cortex (CX) (8.79 ± 0.29), medial basal hypothalamus (MBH) (4.34 ± 0.12), dorsal hypothalamus (DH) (8.15 ± 0.46) and preoptic-anterior hypothalamus (POA-AH) (9.01 ± 0.35).

Processing of Tissue and Plasma Samples

Brain and pituitary samples were homogenized (20 strokes at 1000 rev./min) with a Teflon pestle milled to a clearance of 0.125 mm between the pestle and glass homogenization tube (as suggested by McEwen and Zigmond [18]). The homogenization solution consisted of 0.32 M sucrose, 1 mM KH_2PO_4 and 1 mM $MgCl_2$ at a pH of 6.5 (Medium N-II-A, [18]). Homogenized samples were aspirated into 1 ml centrifuge tubes together with an additional 200 μl N-II-A wash of the homogenization tube and pestle. Each sample was uniformly dispersed and a 40 μl aliquot taken for protein determination [11]. The tubes were then centrifuged at 2°C for 10 min at 850 × g. The supernatant fractions were aspirated into 12 × 75 mm test tubes, while the pellet was resuspended in 200 μl N-II-A and recentrifuged as before. The supernatants were combined with the previous supernatants and the pellets resuspended and recentrifuged as before. The supernatants were pooled as before and the pellets resuspended in 200 μl N-II-A containing 0.25% Triton X-100. After a 10 min incubation in ice, Triton-treated pellets were centrifuged for 10 min at 850 × g. The supernatants from the Triton wash were discarded and the pellets (crude nuclear fraction) resuspended in 200 μl N-II-A and transferred to a separate set of 12 × 75 mm test tubes. The centrifuge tubes were washed with an additional 200 μl N-II-A and the washes combined with the previous nuclear fractions.

The crude nuclear and supernatant fractions were then extracted 4 times with 1 ml of the toluene based scintillation fluid. The extracts were pipeted directly into scintillation vials followed by an additional 5 ml of scintillation fluid. The scintillation fluid consisted of PPO (2,5-diphenyl-oxazole) and POPOP (2(4-methyl-5-phenyl-oxazoyl) benzene) (Amersham/Searle, Arlington Heights, IL) dissolved in analytical grade toluene at concentrations of 5.0 and 0.05 g/l., respectively. Plasma samples were processed in the same manner as supernatant fractions. The radioactivity levels were determined in a Packard Model 2405 liquid scintillation spectrometer. All samples were counted for 20 min each and all data converted to disintigrations per min (DPM) by an external standard channels ratio method. This method revealed that our counting efficiency was approximately 50% for tritium. All DPM data were normalized by dividing through by tissue weight (DPM/mg tissue) or plasma volume (DPM/μl plasma). Use of protein determinations for the first three samples per group (second half lost due to aberrant standard curve) as a normalization factor failed to alter any of the essential relationships between the samples normalized by either tissue weight or plasma volume.

Procedure

Experiment 1: Interactive effects of DHT with progesterone and DHP on estrogen-induced receptivity. Two weeks after ovariectomy 34 CD-1 female mice were started on a daily SC injection schedule of 2 μg E_2 B/mouse. After 10 days of estrogen priming all females were tested for sexual receptivity and the 24 females displaying the highest L/M scores were selected for further study. Sixteen of these females were given 1 mg DHT/day concurrently with their E_2 B injections, while the remaining 8 females were given 0.1 cc of the benzyl benzoate-oil vehicle (BBO) plus the standard 2 μg E_2 B/day injections. After two days of treatment all females were again tested for sexual receptivity (approximately 24 hr after their last E_2 B+DHT or E_2 B+BBO injections). The DHT and BBO injections were then halted and the females tested after an additional 3 and then 6 days of E_2 B treatment. After this recovery period all females were started on a series of daily SC injections of 1 mg DHT/day concurrent with their standard 2 μg E_2 B/day injections. Females were tested after 2, 8, 10 and 15 days of this injection schedule (all tests approximately 24 hr after the last E_2 B+DHT injections). After the Day 15 test all females were given their daily E_2 B and DHT injections and the 16 females consistently displaying the lowest L/M scores during the last 3 tests were selected for an additional test on the next day. However, unlike all previous tests, SC injections of either 500 μg progesterone (n = 8) or 500 μg DHP (n = 8) were given 6 hr prior to the test. All females were then tested after an additional 3 and then 6 days of E_2 B treatment. The injection and testing schedule used in this experiment is illustrated in Fig. 1.

Experiment 2: Effects of varying dose and time of administration of DHT on estrogen-induced receptivity. Two weeks after ovariectomy 15 CD-1 female mice were started on a daily SC injection schedule of 2 μg E_2 B/mouse. After 10 days of estrogen priming all females were tested for sexual receptivity and the 12 females displaying the highest L/M scores were selected for further study. Half of these females were given 1 mg DHT/day concurrently with their E_2 B injections, while the other half were given their 1 mg DHT/day SC injections 12 hr after the daily E_2 B injections. After 5 days of DHT injections the dose was reduced to 500 μg DHT/day and 9 days later all females were given the BBO vehicle only, either concurrent with, or delayed 12 hr after the E_2 B injections. All females were tested for sexual receptivity after 2, 5, 8, 11, 14, 17 and 21 days of the DHT-BBO injection paradigm. The injection and testing schedule used in this experiment is illustrated in Fig. 2.

Experiment 3: Effects of DHT, CA, and 3α- and 3β-Adiol on estrogen-induced receptivity. Four weeks after ovariectomy 24 CD-1 female mice were started on a daily SC injection schedule of 2 μg E_2B/mouse. After 10 days of estrogen priming all females were tested for sexual receptivity and the 12 females displaying the highest L/M scores were selected for further study. As shown in Fig. 3 these females were divided into two groups of six and started on a series of androgen, anti-androgen or BBO vehicle injections concurrent with the daily E_2 B injections. The dose, sequence and duration of treatment given to Group A were as follows: 1 mg DHT/day × 5 days, 0.2 cc BBO vehicle/day × 2 days, 1 mg 3β-Adiol/day × 8 days, 1 mg 3α-Adiol × 5 days and 1 mg DHT/day × 5 days. These treatments were alternated with 6–8 day periods during which the females received only the E_2 B injections. On the basis of Experiments 1 and 2 this injection schedule of DHT was predicted to be sufficient to inhibit E_2B-induced receptivity. The 3α-Adiol injection schedule was chosen to parallel that used for DHT, while eight rather than five days of 3β-Adiol treatment were given since Baum and Vreeburg [1] had previously shown that 3β-Adiol failed to inhibit E_2 B-induced receptivity in rats. The Group B females received BBO vehicle injections in parallel with all the androgen treatments given to Group A females and a two day sequence of 5 mg CA/day during the period when the Group A females were receiving the BBO vehicle. The CA treatment was stopped after two days since the E_2 B-induced receptivity was clearly already inhibited and since our previous work with rats [17] indicated that the two days of CA treatment (10 mg/day) were sufficient to block receptivity. All females were tested every 2–3 days throughout the entire injection paradigm.

Experiment 4: Effects of DHT and 3α- and 3β-Adiol on ^{3}H-Estradiol localization in brain and pituitary. Ten days after ovariectomy 24 CD-1 female mice were randomly divided into four groups of six mice each. Females in each group received SC injections of 1 mg DHT, 3α- and 3β-Adiol or 0.2 cc of the BBO vehicle. Thirty min later females were anesthetized with methoxyflurane and given a single 40 μl tail vein injection of 20 μCi ^{3}H-estradiol. Sixty min later mice were again anesthetized with methoxyflurane, and brain, pituitary and plasma samples removed and processed as described before. The logistics were designed so that one mouse from each group was processed before processing the second mouse of a group, and so on until all of the mice were processed.

RESULTS

Experiment 1

The L/M scores observed during each of the tests are illustrated in Fig. 1. The first two day sequence of concurrent DHT injections was found to dramatically reduce the L/M scores when these females are compared to either the BBO-treated females, $t(22) = 6.01$, $p<0.01$ or to

their last E_2 B-only test two days earlier, $t(15) = 11.4$, $p<0.01$. The BBO treatment failed to alter the L/M scores ($p>0.1$). Within 3 days after cessation of the DHT injections the L/M scores of these females had significantly improved, $t(15) = 6.02$, $p<0.01$, such that they were now equivalent to those displayed during the first E_2 B-only test.

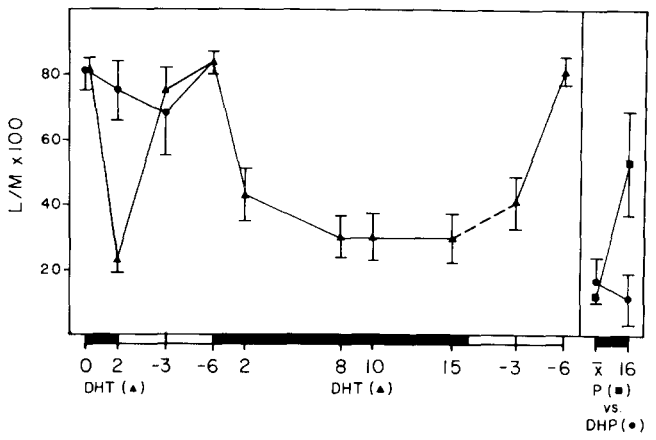

FIG. 1. Lordosis quotients ($\bar{X} \pm$ SEM) for E_2 B-primed ovariectomized mice. Solid bars along the abscissa indicate periods (days) during which either DHT (triangles) or BBO-vehicle (circles) was given concurrently with E_2 B. Only E_2 B was given during the periods with open bars. Panel on right illustrates the effects of either progesterone (squares) or DHP (circles) priming on Day 16 of DHT treatment on sexual receptivity in the 16 E_2 B-primed females displaying the consistently lowest L/M scores during the tests after 8, 10 and 15 days of DHT treatment ($\bar{X}$ = mean of these three tests). Numbers on abscissa refer to either the number of days of DHT treatment (positive numbers) or to the number of days after cessation of such treatment (negative numbers).

Within two days after initiating the second sequence of DHT injections the L/M scores were again significantly reduced, $t(23) = 5.37$, $p<0.01$, and reached stable low values during the tests after 8–15 days of concurrent DHT treatment. Pretreatment with progesterone after 16 days of DHT injections significantly improved the L/M scores compared to those displayed on the previous day, $t(7) = 3.12$, $p<0.025$, while pretreatment with the same dose of DHP failed to alter the DHT-reduced L/M scores. Within six days after cessation of the concurrent DHT injections the L/M scores of all females had increased significantly compared to the values observed after 15 days of DHT treatment, $t(23) = 7.30$, $p<0.01$. The L/M scores on this last test were equivalent to those displayed during the first E_2 B-only test.

Experiment 2

The L/M scores observed during this series of tests are illustrated in Fig. 2. The L/M scores in both the concurrent, $t(5) = 11.18$, $p<0.01$, and 12 hr-delayed, $t(5) = 2.65$, $p<0.05$, DHT treatment groups were significantly reduced after the first two days of DHT treatment compared to the E_2 B-only test. However, the L/M scores in the concurrent treatment groups were also significantly lower than the L/M scores in the delayed treatment group, $t(10) = 2.29$, $p<0.05$. Three more days of DHT treatment produced even lower L/M scores such that the difference between the concurrent and delayed DHT treatment group scores now only approached significance, $t(10) = 1.89$, $p<0.1$. After reducing the daily DHT dose from 1 mg to 500 μg, the L/M scores in the delayed treatment group increased dramatically, while those in the concurrent treatment group failed to improve. After nine days of treatment with the lower dose of DHT the L/M scores in the delayed treatment group were significantly higher than those in the concurrent treatment group, $t(10) = 2.93$, $p<0.025$, and they were not significantly different from those displayed during the first E_2 B-only test. Within three days after cessation of the DHT injections the concurrent treatment group (now receiving daily BBO-vehicle injections) showed a significant improvement in their L/M scores, $t(5) = 6.74$, $p<0.01$, such that they then displayed receptivity scores equivalent to those observed in the first E_2 B-only test. The small improvement in the L/M scores in the delayed treatment group following cessation of the DHT injections was not significant.

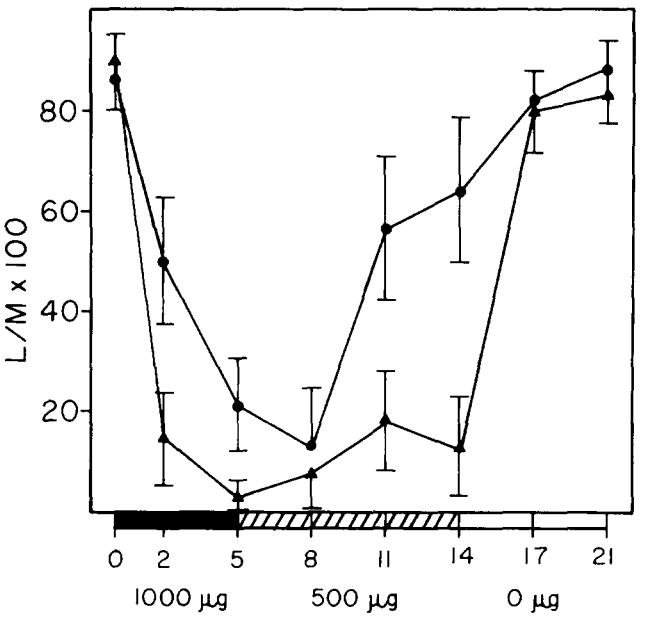

FIG. 2. Lordosis quotients ($\bar{X} \pm$ SEM) for E_2 B-primed ovariectomized mice receiving DHT injections (1000 μg/day, 500 μg/day or 0 μg/day) either concurrent with (triangles), or delayed 12 hr after (circles), the E_2 B injections.

Experiment 3

The L/M scores displayed during this last series of behavioral tests are illustrated in Fig. 3. As in the previous experiments, concurrent treatment with DHT for 2, and especially for 5 days, significantly reduced the L/M scores in the E_2 B-treated females when compared either to their first E_2 B-only test, $t(5) = 6.15$, $p<0.01$, or to the BBO-treated females, $t(10) = 2.83$, $p<0.025$. Within three days after cessation of DHT injections the L/M scores of these females had significantly improved, $t(5) = 6.58$, $p<0.01$, to levels of receptivity equivalent to those displayed during the first E_2 B-only test. Two days of treatment with CA significantly reduced the L/M scores compared to either their last E_2 B-only test, $t(5) = 2.64$, $p<0.05$, or to the BBO-treated females, $t(10) = 6.92$, $p<0.01$. Recovery of sexual receptivity after cessation of the CA injections was

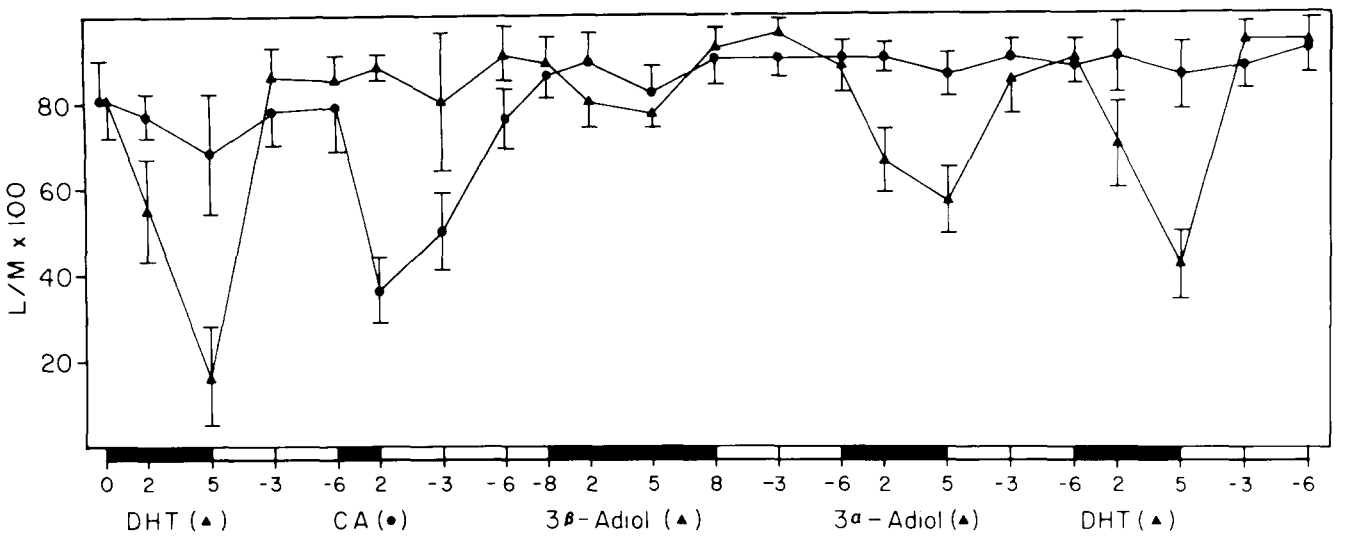

FIG. 3. Lordosis quotients ($\bar{X} \pm$ SEM) for E_2B-primed ovariectomized mice. Solid bars along the abscissa indicate periods during which DHT, cyproterone acetate (CA), 3β-Adiol or 3α-Adiol were given concurrently with E_2B. Controls received BBO-vehicle during these periods. Only E_2B was given during the periods with open bars. Numbers on abscissa refer to either the number of days of androgen or anti-androgen treatment (positive numbers) or to the number of days after cessation of such treatment (negative numbers).

somewhat prolonged in that it required six days rather than the usual three days in order to get a significant improvement of L/M scores, $t(5) = 4.22$, $p<0.01$ to levels similar to those observed in the last E_2 B-only test prior to the CA injections.

Concurrent treatment of E_2 B-primed females with either 3α- or 3β-Adiol produced markedly different effects on receptivity. Up to 8 days of treatment with 3β-Adiol failed to alter L/M scores, while after 2, and especially after 5 days, of treatment with 3α-Adiol L/M scores were significantly reduced compared to both the last pre-3α-Adiol E_2 B only test, $t(5) = 6.53$, $p<0.01$, and to the BBO-treated females, $t(10) = 3.54$, $p<0.01$. Receptivity scores significantly recovered, $t(5) = 3.71$, $p<0.01$, within three days after cessation of the 3α-Adiol injections. The final sequence of DHT injections again produced a consistent reduction in receptivity scores such that by 5 days of treatment the L/M scores were significantly lower than those observed in the last E_2 B-only test prior to starting the DHT injections, $t(5) = 4.50$, $p<0.01$, and to those displayed by the BBO-treated females, $t(10) = 4.02$, $p<0.01$. Receptivity levels recovered, $t(5) = 5.36$, $p<0.01$, to pre-androgen treatment levels within 3 days after cessation of the DHT injections.

Experiment 4

The retention of toluene extractable radioactivity in the crude nuclear and supernatant fractions from the four brain regions, the pituitary and from blood plasma is illustrated in Fig. 4. In the BBO-vehicle treated mice ^{3}H-estradiol accumulation in the pituitary nuclear and supernatant fractions clearly exceeded that found in all brain samples ($p<0.001$, F tests). Within the brain crude nuclear fractions from the BBO vehicle treated females a significant overall single factor analysis of variance, $F(3,15) = 21.55$, $p<0.001$, followed by a Newman-Keuls *a posteriori* test for all possible comparisons [29] revealed that ^{3}H-estradiol accumulation in the MBH and POA-AH was significantly greater than that in the DH and CX brain samples ($p<0.01$). Similarly the brain supernatant fractions from BBO-vehicle treated females displayed a significant overall effect $F(3,15) = 16.34$, $p<0.001$, and subsequent Newman-Keuls tests indicated that ^{3}H-estradiol accumulation in the MBH samples exceeded that found in all other samples ($p<0.01$). ^{3}H-estradiol accumulation in the POA-AH supernatant fractions was also significantly greater than that found in the CX ($p<0.01$), which in turn was greater than that found in plasma ($p<0.05$). The magnitude of the regional differences in the brain crude nuclear fractions clearly exceeded those found in the brain supernatant fractions.

Pretreatment with the various 5α-reduced androgens was found to have no significant effect on ^{3}H-estradiol accumulation in crude nuclear and supernatant fractions from pituitary. Similarly in the brain crude nuclear fractions, the only significant effect was a 25% reduction in radioactivity in the MBH samples following pretreatment with DHT ($t(20) = 2.75$, $p<0.05$; Dunnett's t [29]). There were no significant androgen-pretreatment effects in the brain supernatant fractions, but in the plasma samples 3β- ($t(20) = 4.02$, $p<0.05$; Dunnett's t) and especially 3α-Adiol ($t(20) = 3.20$, $p<0.05$; Dunnett's t) pretreatment resulted in significantly lower DPM/μl values.

DISCUSSION

The present series of experiments has conclusively shown that in ovariectomized CD-1 mice the stimulation of sexual receptivity with chronic daily administration of E_2 B can be effectively blocked with concurrent administration of DHT. Furthermore, in Experiment 1 we found that SC administration of progesterone, but not DHP successfully restored receptivity in these DHT-inhibited females. Thus our previous observations [13] that receptivity in ovariectomized CD-1 mice is blocked by administration of DHT concurrent with E_2 B and DHP, but not with E_2 B and progesterone are consistent with the present results. However, in that former study we concluded that since the E_2 B therapies were the same in both groups, DHT inhibited the display of receptivity by specifically blocking the facili-

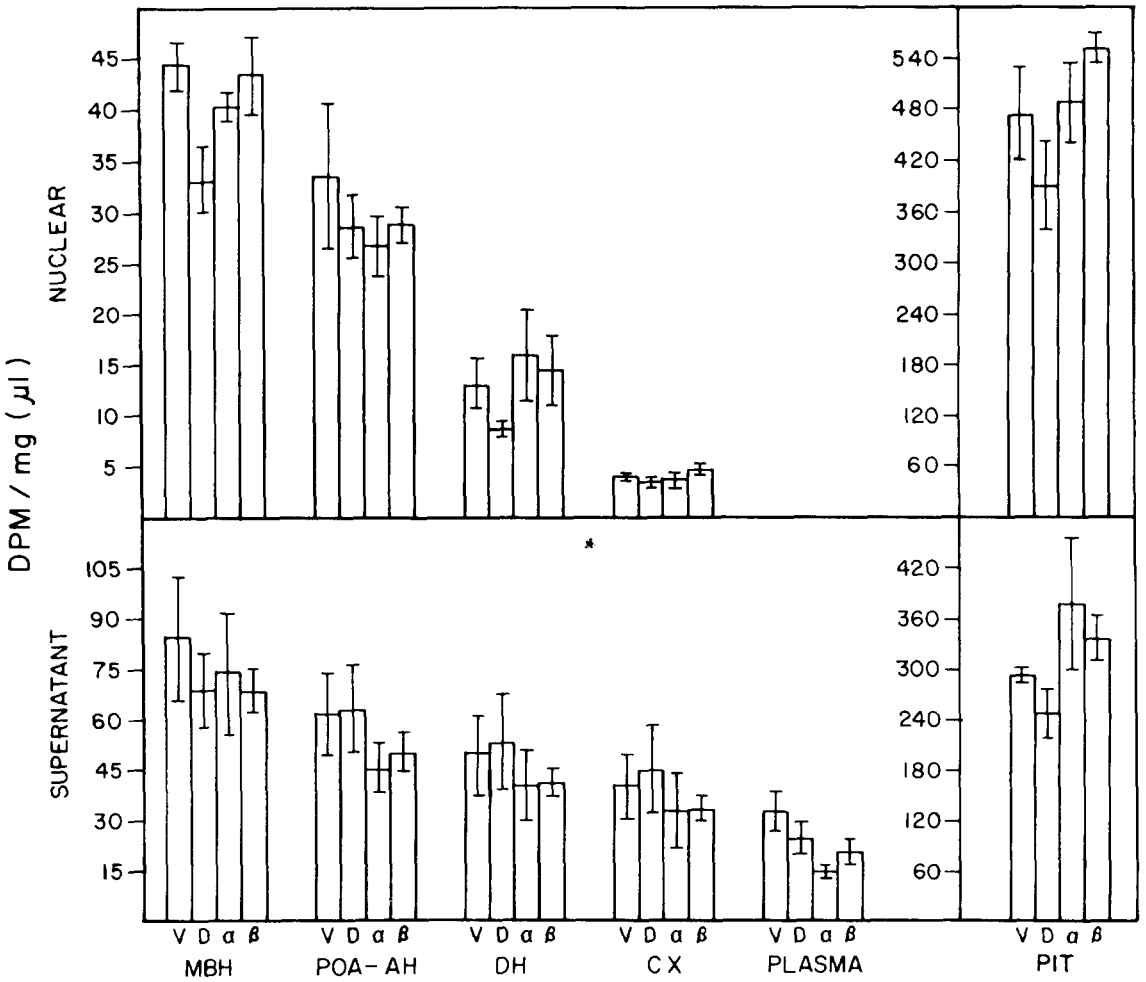

FIG. 4. Effects of pretreatment with BBO-vehicle (V), DHT (D), 3α-Adiol (α), or 3β-Adiol (β) on ^{3}H-estradiol accumulation in crude nuclear and supernatant fractions from medial basal hypothalamus (MBH), preoptic-anterior hypothalamus (POA-AH), dorsal hypothalamus (DH), cerebral cortex (CX) and pituitary (PIT). These data as well as those for blood plasma are expressed as $\bar{X} \pm$ SEM DPM/mg tissue or DPM/μl plasma.

tatory actions of DHP. It now appears that the DHT inhibitory effects are more complex and that they clearly involve anti-estrogenic actions. DHT has also been recently shown to inhibit estrogen-induced sexual receptivity in rats [1] and estrogen plus progesterone-induced receptivity in guinea pigs (Bridson and Goy, personal communication).

The demonstration that progesterone administration 3–6 hr prior to testing is able to at least partially overcome DHT-inhibition of estrogen-induced receptivity in both mice (Experiment 1) and rats [1] adds support to the hypothesis that the mechanisms for estrogen-induced vs. estrogen plus progesterone-induced receptivity are different. For example, Espino *et al.* [8] have recently shown that systemic treatment with the serotonergic agonist, α-methyltryptamine, 30 min prior to testing will block receptivity in female rats treated with E_2 B 48 hr and progesterone 3 hr prior to testing, while it has no effect on receptivity in females receiving chronic E_2 B treatment. Modianos *et al.* [19] have further claimed that receptivity in rats primed with E_2 B and progesterone can be blocked with electrolytic lesions in the habenula, while this treatment fails to reduce estrogen-induced receptivity. Thus even though the inhibitory effects of DHT appear to be opposite to those of α-methyltryptamine administration and habenular lesions, all of these manipulations differentially block either estrogen alone- or estrogen plus progesterone-induced receptivity. The latter two procedures appear to be specifically block the facilitatory actions of progesterone, while DHT appears to act primarily by blocking estrogen actions. However, since progesterone by itself fails to stimulate receptivity in ovariectomized mice and rats [7,20], and since DHT did not completely block receptivity in estrogen-primed females receiving progesterone ([1], Experiment 1), it is also apparent that at the doses tested DHT did not block all of the behavioral actions of E_2 B. Whether or not even higher doses of DHT could totally block all of the behavioral actions of E_2 B remains to be tested.

The mechanism whereby DHT inhibits estrogen-induced sexual receptivity is unclear. In Experiment 2 we found that if the dose of DHT was reduced to 500 μg/day and the injections delayed for 12 hr after the E_2 B injections, then receptivity was not blocked even though this dose of DHT was fully capable of inhibiting receptivity when given

concurrently with the E_2 B. Bridson and Goy (personal communication) have also found that the effectiveness of DHT in inhibiting receptivity in female guinea pigs is inversely proportional to the time of administration after E_2 B. Both of these findings suggest that DHT inhibits some immediate or short latency action of E_2 B. Our findings in Experiment 4 suggest that it is unlikely that DHT inhibits the behavioral actions of E_2 B by blocking estrogen uptake and binding in the brain. Vreeburg *et al.* [24] have further shown that in vitro concurrent incubation of brain cytosol fractions with ^{3}H-estradiol and DHT fails to reduce macromolecular estrogen binding. In retrospect, had DHT blocked estrogen uptake and binding in the brain, it would have been difficult to explain how receptivity was stimulated in the E_2 B plus progesterone situation. It thus appears that DHT inhibited the induction of sexual receptivity in our female mice by blocking some, but not all of the postbinding molecular actions of estrogen in the brain.

In Experiment 3 we found that like DHT, cyproterone acetate inhibited estrogen-induced sexual receptivity when administered concurrently with the daily E_2 B injections. These findings are consistent with our earlier observations in the rat [17]. In addition, we have previously shown that cyproterone acetate pretreatment fails to inhibit ^{3}H-estradiol accumulation in female mouse brain nuclear fractions, whereas the anti-estrogens CI-628 and CN-69,725–27 produce dramatic reductions in ^{3}H-estradiol nuclear binding in the MBH and POA-AH brain regions [16]. Thus, while the anti-estrogens CI-628 and CN-69,725–27 may inhibit the induction of sexual receptivity by inhibiting estradiol cytosol and nuclear receptor binding [12, 16, 17], or by retarding estradiol cytosol receptor replenishment [27,28], the anti-androgen cyproterone acetate appears to block receptivity by inhibiting some behaviorally important postbinding action(s) of estradiol in a similar fashion to that discussed earlier for DHT. The similarity between the behavioral actions of cyproterone acetate and DHT extend to studies on male sexual behavior in rats and mice. For example, in one study we demonstrated that like testosterone and DHT, cyproterone acetate replacement therapy was completely effective in maintaining copulatory behavior in Swiss-Webster mice following castration even though this anti-androgen blocked the stimulatory actions of these androgens on peripheral target tissues [10]. Since cyproterone acetate has also been shown to compete with DHT for binding sites in the brain [22], it is possible that in the rat [17] and mouse (Experiment 3) cyproterone acetate may inhibit estrogen stimulation of sexual receptivity by mimicking the molecular actions of DHT.

Concurrent administration of E_2 B and 3α-, but not 3β-Adiol, also inhibited the display of receptivity in the present study on mice (Experiment 3) and in the Baum and Vreeburg [1] study on rats. In this latter study it was further shown that progesterone administration 4–5 hr prior to testing could partially override the inhibitory actions of 3α-Adiol. Experiment 4 revealed that pretreatment with neither 3α- nor 3β-Adiol had any effect on ^{3}H-estradiol accumulation in any of the brain crude nuclear fractions in spite of the fact that these androgens produced a significant reduction in radioactivity levels in blood plasma. In in vitro studies Vreeburg *et al.* [24] found that 3β-, but not 3α-Adiol inhibited macromolecular ^{3}H-estradiol binding in brain cytosol fractions. Regardless of the apparent differences between these two studies pertaining to 3β-Adiol inhibition of ^{3}H-estradiol binding and to the possible reduction in plasma concentrations in ^{3}H-estradiol following 3α- and 3β-Adiol pretreatment, it is clear that the reduction of estrogen uptake and binding in the brain is not the mechanism of 3α-Adiol inhibition of estrogen-induced receptivity.

It is entirely possible that the inhibitory actions of 3α-Adiol on estrogen-induced receptivity are due to its metabolic conversion to DHT. In support of this hypothesis it has been demonstrated that peripheral androgen target tissues such as the seminal vesicles and prostate readily convert 3α-, but not 3β-Adiol into DHT [3,21]. In terms of growth stimulating potency in peripheral tissues, we have shown that castrate male mouse seminal vesicle growth is stimulated to the greatest extent by DHT, less so by 3α-Adiol ($p<0.05$) and even less so by 3β-Adiol ($p<0.05$) [14]. A similar potency sequence has been demonstrated in gonadotropin negative feedback studies in both rats [23] and mice [14]. Close examination of the data presented in Fig. 3 suggests that the DHT>3α Adiol>3β-Adiol potency sequence may also be valid in comparisons of the relative abilities of these androgens to inhibit estrogen-induced receptivity.

In summary, the present series of experiments has suggested that DHT inhibits estrogen-induced sexual receptivity in the ovariectomized CD-1 mouse by preventing necessary actions of estrogen which occur within the first 12 hr after estrogen administration. Thus DHT-treatment may serve as a useful probe in future experiments examining the molecular mechanisms whereby estrogen induces receptivity. Our data further suggest that 3α-Adiol and cyproterone acetate inhibit estrogen-induced receptivity by either metabolic conversion to DHT or by directly mimicking the molecular actions of DHT. The present data also add further support to the hypothesis that there are different molecular mechanisms whereby sexual receptivity is stimulated by chronic estrogen treatment, estrogen plus progesterone and estrogen plus DHP.

REFERENCES

1. Baum, M. J. and J. T. M. Vreeburg. Differential effects of the anti-estrogen MER-25 and of three 5α-reduced androgens on mounting and lordosis behavior in the rat. *Hormones Behav.* **7**: 87–104, 1976.
2. Beach, F. A. Importance of progesterone to induction of sexual receptivity in spayed female rats. *Proc. Soc. exp. Biol. Med.* **51**: 369–371, 1942.
3. Becker, H., E. G. Grabosch, C. Hoffmann and K. D. Voigt. Metabolism and mode of action of androgens in target issues of male rats. III. Metabolism of 5α-androstane-3,17-dione, of 5α-androstane-3α,17β-diol and of 5α-androstane-3β,17β-diol in target organs and peripheral tissues. *Acta endocr.* **73**: 407–416, 1973.

4. Boling, J. L. and R. J. Blandau. The estrogen-progesterone induction of mating responses in the spayed female rat. *Endocrinology* **25:** 359–364, 1939.
5. Davidson, J. M., C. H. Rodgers, E. R. Smith and G. J. Bloch. Stimulation of female sex behavior in adrenalectomized rats with estrogen alone. *Endocrinology* **82:** 193–195, 1968.
6. Edwards, D. A. Induction of estrus in female mice: Estrogen-progesterone interactions. *Hormones Behav.* **1:** 299–304, 1970.
7. Edwards, D. A., R. E. Whalen and R. D. Nadler. Induction of estrus: estrogen-progesterone interactions. *Physiol. Behav.* **3:** 29–33, 1968.
8. Espino, C., M. Sano and G. N. Wade. Alpha-methyltryptamine blocks facilitation of lordosis by progesterone in spayed, estrogen-primed rats. *Pharmac. Biochem. Behav.* **3:** 557–559, 1975.
9. Gorzalka, B. B. and R. E. Whalen. Genetic regulation of hormone action: selective effects of progesterone and dihydroprogesterone (5α-pregnane3,20-dione) on sexual receptivity in mice. *Steroids* **23:** 499–505, 1974.
10. Hall, N. R. and W. G. Luttge. Maintenance of sexual behavior in castrate male SW mice using the anti-androgen, cyproterone acetate. *Pharmac. Biochem. Behav.* **3:** 551–555, 1975.
11. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275, 1951.
12. Luttge, W. G. Intracerebral implantation of the anti-estrogen CN-69,725-27: Effects on female sexual behavior in rats. *Pharmac. Biochem. Behav.* **4:** 685–688, 1976.
13. Luttge, W. G. and N. R. Hall. Interactions of progesterone and dihydroprogesterone with dihydrotestosterone on estrogen activated sexual receptivity in female mice. *Hormones Behav.* **7:** 253–257, 1976.
14. Luttge, W. G., N. R. Hall and C. J. Wallis. Studies on the neuroendocrine, somatic and behavioral effectiveness of testosterone and its 5α-reduced metabolites in Swiss-Webster mice. *Physiol. Behav.* **13:** 553–561, 1974.
15. Luttge, W. G., E. G. Grant, Jr. and H. E. Gray. Regional and subcellular localization of [^{3}H]dihydrotestosterone in brain and pituitary of male mice. *Brain Res.* **109:** 426–429, 1976.
16. Luttge, W. G., H. E. Gray and J. R. Hughes. Regional and subcellular [^{3}H]estradiol localization in selected brain regions and pituitary of female mice: Effects of unlabeled estradiol and various anti-hormones. *Brain Res.* **104:** 273–281, 1976.
17. Luttge, W. G., N. R. Hall, C. J. Wallis and J. C. Campbell. Stimulation of male and female sexual behavior in gonadectomized rats with estrogen and androgen therapy and its inhibition with concurrent anti-hormone therapy. *Physiol. Behav.* **14:** 65–73, 1975.
18. McEwen, B. S. and R. E. Zigmond. Isolation of brain cell nuclei. In: *Research Methods in Neurochemistry*, edited by N. Marks and R. Rodnight. New York: Plenum Press, 1972.
19. Modianos, D. T., J. C. Hitt and H. B. Papolow. Habenular lesions and feminine sexual behavior of ovariectomized rats: Diminished responsiveness to the synergistic effects of estrogen and progesterone. *J. comp. physiol. Psychol.* **89:** 231–237, 1975.
20. Ring, J. R. The estrogen-progesterone induction of sexual receptivity in the spayed female mouse. *Endocrinology* **34:** 269–275, 1944.
21. Schmidt, H., O. Giba-Tziampiri, G. V. Rotteck and K. D. Voigt. Metabolism and mode of action of androgens in target tissues of male rats. IV. Mode of action of 5α-androstane-3,17-dione, 5α-androstane-3α,17β-diol and of 5α-androstane-3β,17β-diol at a cellular level on seminal vesicles and prostates of rats. *Acta Endocr.* **73:** 599–611, 1973.
22. Stern, J. M. and A. J. Eisenfeld. Distribution and metabolism of ^{3}H-testosterone in castrated male rats; effects of cyproterone, progesterone and unlabeled testosterone. *Endocrinology* **88:** 1117–1125, 1971.
23. Swerdloff, R. S., P. K. Grover, H. S. Jacobs and J. Bain. Search for a substance which selectively inhibits FSH – effects of steroids and prostaglandins on serum FSH and LH levels. *Steroids* **21:** 703–722, 1973.
24. Vreeburg, J. T. M., P. J. M. Schretlen and M. J. Baum. Specific, high-affinity binding of 17β-estradiol in cytosols from several brain regions and pituitary of intact and castrate adult male rats. *Endocrinology* **97:** 969–977, 1975.
25. Whalen, R. E. Estrogen-progesterone induction of mating in female rats. *Hormones Behav.* **5:** 157–162, 1974.
26. Whalen, R. E. and B. B. Gorzalka. The effects of progesterone and its metabolites on the induction of sexual receptivity in rats. *Hormones Behav.* **3:** 221–226, 1972.
27. Whalen, R. E. and B. B. Gorzalka. Effects of an estrogen antagonist on behavior and on estrogen retention in neural and peripheral target tissues. *Physiol. Behav.* **10:** 35–40, 1973.
28. Whalen, R. E., J. V. Martin and K. L. Olsen. Effect of oestrogen antagonist on hypothalamic oestrogen receptors. *Nature* **258:** 742–743, 1975.
29. Winer, B. J. *Statistical Principles in Experimental Design.* New York: McGraw-Hill, 1962.