Pergamon

Physiology & Behavior, Vol. 57, No. 2, pp. 307–313, 1995

0031-9384/95 $9.50 + .00

0031-9384(94)00247-9

Progesterone Modulation of Androgen-Dependent Sexual Behavior in Male Rats

DIANE M. WITT,*[1] LARRY J. YOUNG† AND DAVID CREWS†

*Laboratory of Neurophysiology, National Institute of Mental Health, Poolesville, MD 20837 and †Institute of Reproductive Biology and Department of Zoology, University of Texas at Austin, Austin, TX 78712

Received 22 December 1993

WITT, D. M., L. J. YOUNG AND D. CREWS. *Progesterone modulation of androgen-dependent sexual behavior in male rats.* PHYSIOL BEHAV **57**(2) 307–313, 1995.—The present study examines the effects of physiological levels of progesterone (P) on copulatory behavior in sexually naive male rats. Two weeks after gonadectomy males were implanted with either empty Silastic capsules (BL) or Silastic capsules containing testosterone (T), P, or both (P + T). When tested with an estrous female, all of the gonadally intact males (intact) and none of the BL controls exhibited mounting/intromission behaviors. Mounting was observed in 75% of the T-alone males. More than half (64%) of the P-alone males and 100% P + T males exhibited mounting. In most cases, mounting was followed by intromission responses. Subsequently, intact and gonadectomized males received daily injections of the P antagonist RU486 along with hormone treatment. After receiving RU486, only 63% of the intact males and 71% of the T-alone males mounted successfully. The facilitatory effects of P on copulatory behavior were completely abolished by RU486 treatment. The present studies provide the first evidence in mammals suggesting that P-dependent mechanisms influence neurochemical pathways involved in copulation.

RU486 Copulation Corticosterone Testosterone Progesterone

A VAST literature exists documenting the effects of progestins (P) on sexual behavior in female mammals. Essentially, P facilitates lordosis and is required for the normal expression of proceptivity (solicitation of the male) as well as sexual receptivity in estrogen-primed females (12,35). The actions of P are thought to be exerted by genomic mechanisms acting via estrogen-inducible P receptors localized in the medial preoptic area (MPOA) and ventromedial nucleus of the hypothalamus (VMN) (5,6,32,33,36,37), brain regions known to regulate female reproductive behavior [reviewed in (26)]. However, in other brain areas P-dependent mechanisms may enhance sexual behavior through nongenomic actions on neural tissue (8,13,23). These findings have prompted a reexamination of the physiological role of P in the expression of sexual behavior in females.

The physiological role of P in modulation of androgen-dependent sexual behavior in males is less clearly understood. Numerous studies have established that testicular testosterone (T) and the MPOA, where T is intracellularly converted to other androgens and estradiol (30), are essential for the full expression of masculine sexual responses in many species [reviewed in (31)]. Pharmacological dosages of P have inhibitory effects on androgen-dependent sexual behavior in numerous species, including guinea pigs (7), mice (11), quail (3), ring doves (9), pigeons (10), and nonhuman primates (2). Although progesterone's actions are still unclear in humans, progestin therapy has been routinely used as a means to control the libido of felony sex offenders (4,20). Mechanisms proposed for these antiandrogenic effects of pharmacological doses of P include decreased uptake of androgen in target tissues (34), increased androgen catabolism in the liver (1), inhibition of T reduction to dihydrotestosterone in target tissues (24), and interference with androgen receptor mechanisms (7). In these studies, injections of large doses of P produced supraphysiological concentrations of circulating P. Therefore, definitive conclusions cannot be drawn from their results regarding the role of endogenous P and its physiological effects on the sexual behavior of males.

Evidence that progestins may stimulate masculine sexual behavior in vertebrates was initially discovered in *Cnemidophorus uniparens*, the all-female species of parthenogenetically reproducing whiptail lizards, and was later extended to males of two other sexually reproducing species. With the absence of males, *C. uniparens* parthenogens alternate between expressing feminine sexual receptive behavior during vitellogenisis and masculine mounting and copulatory behavior following ovulation. Elevated plasma levels of P are associated with ovulation and facilitate this expression of male-like pseudosexual behaviors (15). In little striped whiptail lizards (*C. inornatus*), a sexual ancestor of *C. uniparens*, androgens regulate mounting and copulatory behaviors, but exogenous P can also restore the full complement of sexual responses in some gonadectomized males (21). These progestagenic effects are blocked by concomitant administration of the synthetic P antagonist RU486 and are mimicked

[1] Requests for reprints should be addressed to Diane M. Witt, Ph.D., Laboratory of Neurochemistry, NINDS, NIH, Bldg. 36, Room 4D10, 9000 Rockville Pike, Bethesda, MD 20892.

with the nonmetabolizable progestin agonist R5020 (22). Low dosages of P, acting synergistically with subthreshold levels of androgens such as DHT, reinstate courtship behavior in castrated *C. inornatus* males (22). As in the rat, both androgen and progesterone receptors are concentrated in the MPOA of both the sexual and unisexual species (39). In an unrelated lizard species, the green anole (*Anolis carolinensis*), high levels of P will inhibit sexual behavior in intact males, whereas lower levels of P act synergistically with exogenous androgens to reinstate courtship behavior in gonadectomized males (38).

The paradoxical effects of P, stimulation/inhibition of masculine sexual behavior, suggest two hypotheses: (i) the neuroendocrine pathways underlying P facilitation of masculine sexual behavior are not unique to *Cnemidophorus* spp., and (ii) pharmacological doses of P that reportedly produce antiandrogenic effects in other vertebrates may not accurately reflect the physiological role of P in androgen-dependent sexual behavior. Behavioral responses to P may differ as a function of dosage, route, or frequency of administration, or interspecies variation of brain mechanisms involved in the expression of these behavioral responses. The findings from research using reptilian species prompted us to reevaluate the physiological role of P in facilitating androgen-dependent sexual behavior in mammalian species. The present studies examine the effect of physiological levels of P (delivered via Silastic capsules) in gonadectomized male rats to assess the role of this steroid in the initiation of reproductive behavior in sexually naive males. We tested whether P could directly facilitate/initiate sexual behavior without prior exposure to: 1) mating experiences and/or learned behavioral responses, or (2) elevated T levels associated with previous copulatory interactions. In addition, behavioral effects were studied in both gonadally intact and gonadectomized/hormone-supplemented male rats receiving daily injections of the progestin receptor antagonist (RU486). All behaviors were compared to those responses typically observed in gonadally intact/sexually naive male rats.

METHOD

Animals

Adult male Sprague–Dawley rats (Taconic Farms, Germantown, NY), weighing 350–400 g, were maintained in individual cages on a 14:10 light:dark cycle, with lights out at 1200 h. Food and tap water were available ad lib. Hormone-primed estrous females were used as sexual stimuli for males in all behavioral tests. Sexually naive males remained gonadally intact (intact) or were gonadectomized while under chloropent anesthesia.

Hormone Treatment: Experiments 1 and 2

After a 2-week recovery period, the animals were again anesthetized and implanted (SC, dorsal thoracic level) with Silastic capsules prepared as described in (21). Silastic capsules were packed with crystalline T (30 mm, i.d. 0.078, o.d. 0.125) or P (10 mm, i.d. 0.04 in., o.d. 0.85 in.). Blank (BL) capsules, without hormones, of the appropriate size served as controls. Each animal was implanted with two capsules (either one T or a 30-mm BL and one P or a 10-mm BL). Animals in Experiment 2 received further manipulation (see below).

Injection Procedures: Experiment 2

On the day of capsule implantation males began receiving injections (IP) of the P receptor antagonist RU486 (2 mg/kg/day) (Roussel–Uclaf, Romainville, France) suspended in sesame oil. The RU486 dosage was selected based on doses that do not influence LH surges in gonadally intact animals (19).

Testing Procedures

All behavioral testing was conducted in a clear Plexiglas arena (38 × 44 × 24 cm) 1–2 h into the dark phase of the light:dark cycle. For precise scrutiny of sexual contacts, the arena was raised approximately 20 cm and a mirror (40 × 48 cm) was placed under the arena floor to provide ventral viewing for accurate determination of extra- and intravaginal intromission frequencies and ejaculations (see below). One week after capsule implantation, males were placed in the testing arena and allowed to acclimate for 5 min. Subsequently, a hormonally primed, sexually receptive female was added. All observations were confined to a 15-min maximal testing period. These experimental conditions were based on optimal stimulus requirements for ejaculatory responses typically observed in gonadally intact, sexually naive male rats from our colony. The estrous females were replaced with fresh stimulus females if the male became disinterested. Up to three fresh stimulus females could be used for any 15-min test session.

Behavioral Measures

All behavioral measures were monitored by a single observer, unfamiliar with the male's hormone treatment, using a computerized event recorder system. Attempted mounts (AT MT) were recorded when the male grasped the female's hind quarters, attempting to make genital contact, without pelvic thrusts. Mounting (MT) was recorded when the male's forelimbs grasped the female's hind quarters and repeated pelvic thrusting was directed towards the female's genital region. Intromissions (INTRO) were recorded only when penile insertion into the vagina was verified by ventral viewing. Ejaculation (EJAC) was recorded when the male exhibited an ejaculatory pattern (spasmodic contractions of skeletal muscles such as hips, hindlimbs, and forelimbs) during genital contact (intromissions) preceded by repeated pelvic thrusting. Autogrooming (GROOM) occurred when males rhythmically passed their forelimbs over their bodies.

At the close of the testing period males were returned to their home cages. Approximately 18 h (0900–1000) after the onset of testing, body weights were determined and males were euthanized. Trunk blood samples were collected into heparinized tubes (kept on ice until centrifugation) and seminal vesicles were dissected, emptied, and wet weight measurements taken as a bioassay of androgen stimulation.

Radioimmunno Assay of Hormones

After centrifugation, plasma was collected and frozen at −20°C until each sample was assayed for P, T, and corticosterone (B) levels at the close of Experiment 2. For T, 100 μl of plasma was extracted with 3 ml of diethyl ether and dried under a stream of nitrogen and resuspended overnight in assay buffer. Each sample was assayed in duplicate using a standard tritiated radioimmunoassay technique utilizing a T antibody (Wein Laboratories, Succasunna, NJ) with a cross-reactivity of 65% with DHT and $< 0.2\%$ with P. Because our P antibody (generous gift of G. Niswinder, Colorado State University) has a cross-reactivity of 2.2% with B (14), it was necessary to use a hexane extraction of the samples to remove any corticosterone that could interfere with the assay. For the progesterone assay, 100 μl of plasma was extracted with 3 ml of hexane. The hexane extract was then back extracted with 500 μl of water to remove any residual B. The hexane extract was removed after freezing the aqueous layer in a dry ice/methanol bath. Subsequently, the hexane extract was dried under a stream of nitrogen after which the samples were suspended in the assay buffer overnight. This procedure removed

99.3% of the B from the sample. Progesterone levels were determined in duplicate for each sample using a standard tritated P radioimmunoassay. B levels were determined using the RSL [^{125}I]corticosterone kit (ICN Biomedicals, Inc., Costa Mesa, CA). All unextracted samples were processed in duplicate in a single assay. Using the provided antibody, the percent cross-reactivity was 0.10% and 0.02% for T and P, respectively.

Statistical Analyses

A one-way analysis of variance (ANOVA), followed by a post hoc Scheffe F-test, was used to determine group differences in seminal vesicle weight and plasma hormone levels. For behavioral measures, within-group differences (in a given experiment) were determined by chi-square tests performed on the percentages of males exhibiting a specific behavior. Group means (based on frequency of a specific behavior) were compared by a one-way ANOVA in groups exhibiting the specific behavior. Where overall differences in group means were significant, post hoc comparisons between group means were made individually using the Scheffe F-test. An alpha < 0.05 was required for significance.

Experiment 1: Effects of Chronic Progesterone on the Initiation of Copulatory Behavior in Sexually Naive Males

Sexually naive males were gonadectomized and 2 weeks later implanted with either two empty Silastic capsules (BL, $n = 6$) or capsules containing hormones such as progesterone only (P, $n = 11$), testosterone only (T, $n = 8$), or P plus T (P + T, $n = 7$). One week later males were paired with estrous females during a 15-min test period and their behavioral responses were compared with the typical sexual interactions seen in gonadally intact males (intact, $n = 7$).

Experiment 2: Copulatory Behavior in Sexually Naive Males Following Progesterone Antagonism

The purpose of the second experiment was to determine whether P or progestin metabolites were modulating the above behavioral responses. Daily injections (IP) of either the P receptor antagonist RU486 (2 mg/kg/day) were given to both gonadally intact (intact) and gonadectomized males (implantated with capsules) starting on the day of capsule implantation. The following groups, I/RU486 ($n = 8$), T/RU486 ($n = 7$), P/RU486 ($n = 8$), B/RU486 ($n = 4$), were subsequently tested using the same behavioral paradigm described in Experiment 1.

RESULTS

Experiments 1 and 2: Physiological Measures

Although biological as well as behavioral data for both Experiment 1 and 2 are presented in the same graph, statistical analyses were performed separately in each experiment, for the purpose of clarity.

Seminal vesicle weights. Among the groups studied, standardized seminal vesicle weights (ratios derived from SV/body weight) differed significantly among males in both Experiment 1, $F(4, 31) = 36.6$, $p < 0.0001$, and Experiment 2, $F(5, 35) = 45.1$, $p < 0.0001$, reflecting the presence or absence of endogenous or exogenous (T capsule) testosterone. In Experiment 1, a significant reduction in SV ratios was observed in males with BL capsules (0.4 ± 0.02) when compared to either intact (1.5 ± 0.1), T-only (1.4 ± 0.08), or P + T males (1.4 ± 0.1) (Scheffe $F = 14.7$, 12.6, and 12.9, $p < 0.05$, respectively). BL males and P males had similar SV ratios. P males also had significantly lower SV ratios relative to intact, T-only, and P + T (Scheffe $F = 18.2$, 15.9, and 15.9, $p < 0.05$, respectively). In Experiment 2, standardized seminal vesicle weights (SV ratios) were significantly lower in all castrate males (P/RU486 = 0.4 ± 0.02; BL/RU486 = 0.4 ± 0.04) when compared to either T/RU486 (1.3 ± 0.1) males (Scheffe $F = 15.1$ and 10.0, $p < 0.05$, respectively) or I/RU486 (1.5 ± 0.04) males (Scheffe $F = 21.1$ and 14.4, $p < 0.05$, respectively). Seminal vesicle weights did not differ between T/RU486 and I/RU486. RU486 treatment itself had no effect on seminal vesicle weights.

Plasma T levels. RIA results for T or P levels reflected the Silastic capsule contents in each animal [Fig. 1(A,B)]. In Experiment 1, significant group differences in mean plasma T levels were found, $F(4, 30) = 5.3$, $p < 0.003$ [Fig. 1(A)]. All castrates without T supplementation had significantly lower plasma T levels than those found in intact controls (Scheffe $F = 3.1$, and P-only, Scheffe $F = 4.0$, $p < 0.05$). As expected, castrates with either T-only or P/T capsules had intermediate, but not significantly different, levels of plasma T when compared to intact controls. Likewise, males with either BL or P-only capsules did not differ significantly in plasma T levels. In Experiment 2, plasma T levels were significantly different, $F(5, 31) = 15.2$, $p < 0.0001$, among males having endogenous and exogenous T exposure relative to castrates with P or BL capsules [Fig. 1(A)]. Significantly

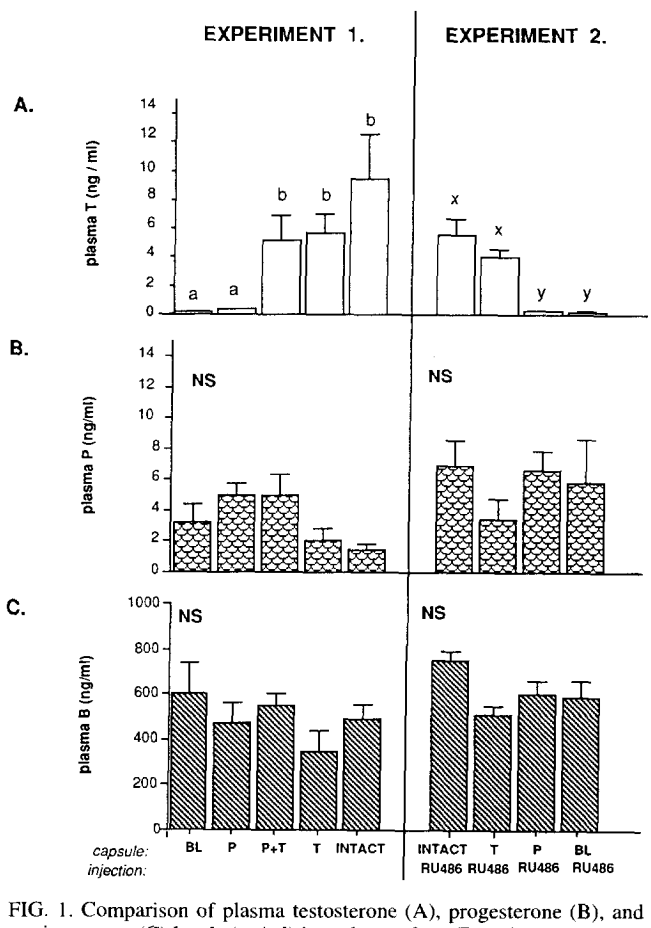

FIG. 1. Comparison of plasma testosterone (A), progesterone (B), and corticosterone (C) levels (ng/ml) in male rats from Experiments 1 and 2. NS denotes no significant differences found between groups within each experiment. In cases where significant differences were found, based on ANOVA, a post hoc Scheffe F-test analysis was performed on data collected within each experiment and groups bearing different letters differed significantly ($\alpha = 0.05$) from each other.

lower levels of plasma T were observed in BL/RU486 males relative to both I/RU486 males (Scheffe $F = 5.8$, $p < 0.05$) and T/RU486 males (Scheffe $F = 2.8$, $p < 0.05$), respectively. Also, plasma T levels were significantly lower in P/RU486 males relative to both I/RU486 (Scheffe $F = 8.7$, $p < 0.05$) and T/RU486 (Scheffe $F = 4.2$, $p < 0.05$), respectively. Plasma T levels were similar in both P and BL males receiving RU486. Intact or T-treated males receiving RU486 had similar plasma T levels.

Plasma P levels. In Experiment 1, mean plasma P levels were significantly different among the groups, $F(4, 30) = 3.7$, $p < 0.01$ [Fig. 1(B)]; however, the Scheffe F-test was too conservative to indicate significance between group differences at the $p < 0.05$ level. There appeared to be a trend towards elevated plasma P levels in males bearing either P-only or P + T capsules. In Experiment 2, plasma P levels were similar in all male groups, but were slightly elevated when compared to plasma P levels in Experiment 1 [Fig. 1(B)]. It should be noted that castration does not completely abolish endogenous P, and our P treatment should be considered a supplementation of the endogenous P levels. These P levels in our intact and castrated males and the increase in P levels following castration are similar to those previously reported for males rats (29).

Plasma B levels. In Experiment 1, plasma B levels were similar across all groups [Fig. 1(C)]. In Experiment 2, levels of B were similar in most groups (not significantly different) and treatment with RU486 did not appear to alter B levels when compared to levels in Experiment 1.

Experiment 1: Behavioral Results

Percent of male rats expressing sexual behavior. All males (100%) exhibited autogrooming (GROOM) behavior that did not differ as a function of hormonal status or sexual activity. Also, the percent of male rats expressing AT MT (75–100%) did not vary significantly as a function of capsule contents [Fig. 2(A)]. Group BL, which consisted entirely of nonmating males, was omitted from subsequent analyses of copulatory behaviors [Fig. 2(B,C)] in groups containing sexually active males (all other groups). There were significant group differences in the percentage of males exhibiting MT behavior, $\chi^2(3) = 11.6$, $p < 0.01$ [Fig. 2(B)]. However, the percentage of males (83–100%) showing INTRO behavior did not differ significantly as a function of hormonal status in sexually active males [Fig. 2(C)]. Only intact males experienced ejaculations.

Specific expression of sexual behavior. AT MTs were exhibited by males in all groups. Although the high rate of variability may have obscured significant group differences, a trend towards elevated AT MT frequencies was apparent in males receiving exogenous P [Fig. 3(A)]. Closer examination revealed no significant differences in the frequency of the expression of MTs [Fig. 3(B)]. Mean intromission (INTRO) frequencies, $F(3, 19) = 3.3$, $p < 0.04$, were significantly altered by capsule contents [Fig. 3(C)]. Mean frequencies of INTRO did not significantly differ between P-only, T-only, and P + T males. Yet, INTRO frequencies were significantly lower in males implanted with P-only capsules, when compared with gonadally intact males (Scheffe $F = 3.14$, $p < 0.05$). All other groups with exogenous or endogenous T showed similar INTRO frequencies. Ejaculatory responses were only observed in the gonadally intact males during the 15-min testing period. Although autogrooming was observed in all males, the frequency of GROOM was significantly different among the groups, $F(4, 34) = 7.6$, $p < 0.0002$ [Fig. 3(D)]. GROOM frequencies were significantly reduced in BL males when compared to either P + T males, T-only males, or intact males (Scheffe $F = 3.0$, 2.9, and 4.2, $p < 0.05$, respectively) [Fig. 3(D)].

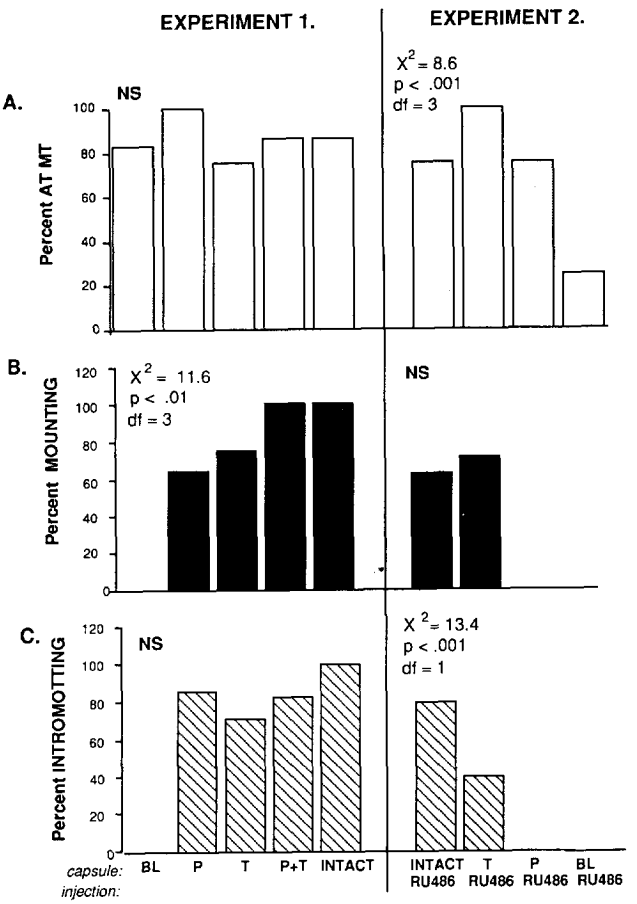

FIG. 2. Percentage of male rats exhibiting attempted mounts (AT MT), actual mounts, and intromissions within each experiment. Chi-square statistic indicates where groups differ significantly from expected values. Only groups with males exhibiting behaviors were included in these analyses.

Experiment 2: Behavioral Results

Percent males expressing sexual behavior. The percentage of males expressing AT MT varied as a function of hormonal status and ligand injection, $\chi^2(3) = 8.6$, $p < 0.001$ [Fig. 2(A)]. There were no significant group differences in the percent of males exhibiting MT behavior [Fig. 2(B)]. The percentage of males showing INTRO behavior differed as a function of hormonal status and ligand injection in sexually active males, $\chi^2(1) = 13.4$, $p < 0.001$ [Fig. 2(C)]. Only Intact males experienced ejaculations. The percent of males (88–100%) exhibiting autogrooming (GROOM) behavior did not differ as a function of hormonal status, injection treatments, or sexual activity.

Specific expression of sexual behavior. AT MTs were observed in males from all groups [Fig. 2(A)]. When this behavior was closely examined there were no significant differences in the frequencies of AT MT among I/RU486, T/RU486, and BL/RU486 [Fig. 3(A)]. However, the P/RU486 group showed significantly higher levels of AT MTs than the T/RU486 (Scheffe $F = 3.27$, $p < 0.05$). P/RU486 and B/RU486 males did not exhibit MT behavior [Fig. 3(B)]. In males continuing to exhibit sexual behavior the frequencies of MTs or INTROs did not differ significantly [Fig. 3(B,C)]. However, both of these behaviors tended to be lower than those typically exhibited by intact and

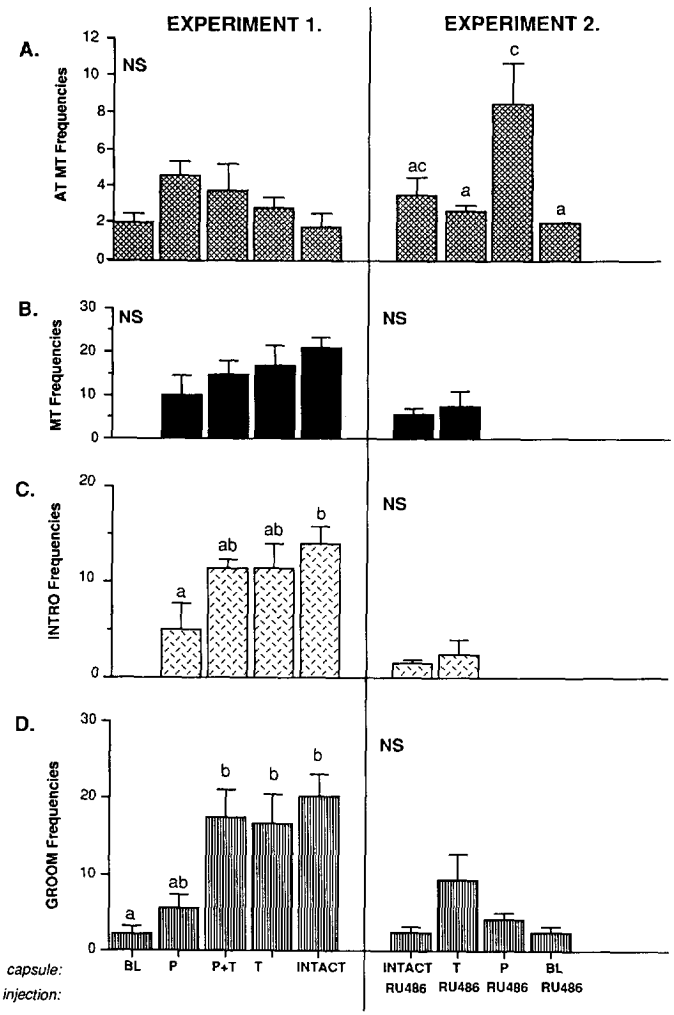

FIG. 3. Frequency measures (mean ± SEM) for attempted mounts (AT MT), actual mounts, and intromissions exhibited by male rats in each experiment. NS denotes no significant differences found between groups within each experiment. In cases where significant differences were found, based on ANOVA, a post hoc Scheffe F-test analysis was performed on data collected within each experiment and groups bearing different letters differed significantly ($a = 0.05$) from each other.

T-only males from Experiment 1. Ejaculatory responses were only observed in the gonadally intact males during the 15-min testing period. Autogrooming was observed in all males, and the mean frequency of GROOM was not significantly different among the groups (Fig. 3D).

DISCUSSION

Previous studies addressing the effects of progestins on androgen-dependent sexual behavior in males used pharmacological doses of P that inhibited sexual responses, therefore providing little information regarding the role of endogenous P in mediating the sexual behavior of males. The conclusions drawn from these experiments were first challenged in studies with reptiles. As mentioned previously, in the parthenogen *Cnemidophorus uniparens* and in two other species of lizards, endogenous or exogenous P serves to facilitate mounting behavior in the absence of androgens. These results prompted us to perform a similar study in rats. This is the first study to date in which the focus has been on the effects of physiological levels of P on sexual behavior in a male mammal.

The present studies demonstrate that supplementation of endogenous P in castrated male rats is capable of initiating the full complement of sexual behavior, even in the absence of other gonadal steroids. Mounting and intromission responses appeared normal. The finding that castrated male rats do not respond uniformly to T treatment is common; that is, some T-treated males will not exhibit sexual behavior when presented with an estrous female. In our study, T alone did not completely restore typical sexual responses in all males unless P levels were elevated. Furthermore, administration of the synthetic antiprogestin RU486 inhibited the expression of sexual behavior, even in some gonadally intact male rats, and completely abolished the facilitatory effects of P supplementation in castrated males.

The most extensively studied P antagonist is RU486 [11β-(4-dimethylamino phenyl)-β-hydroxy-17α-(1-propyl)-4,-9-estradien-3-one. There is evidence that this ligand effectively antagonizes cytosolic progestin receptors and is devoid of agonist activity (27) as determined by in vivo and in vitro models. However, along with antiglucocorticoid effects, RU486 also has moderate antiandrogenic properties (25). Characterization studies of RU486 indicate relative binding affinities (RBA) of 530 for P receptors, 300 for glucocorticoid receptors, and 23 for androgen receptors. RU486 has virtually no interactions with mineralocorticoid or estrogen receptors (25).

RU486 does not inhibit corticosterone synthesis in rat adrenal cells stimulated by ACTH, and exerts moderate antiandrogenic effects on seminal vesicles and prostate weights (28). However, the dosage used in the present study, (2 mg/kg/day), was insufficient for exerting significant antiglucocorticoid or antiandrogenic actions. The antiglucocorticoid effects of RU486 would have resulted in increased release of ACTH followed by increased B levels. Although somewhat variable, there were no differences in B levels in any of our treatment groups. Any antiandrogenic effects of the RU486 treatment would have been apparent by a reduction in the androgen-stimulated weight gain in seminal vesicles. No such differences were apparent in our males. It should also be noted that chronically administered RU486 (15 days) in female rats does not affect ovulatory activity (28). However, these females did exhibit dose-dependent increases in serum LH and progesterone concentrations (28). Similarly, in our experiment all groups receiving RU486 treatment also exhibited increases in plasma P concentrations. These data suggest that under our treatment paradigm using RU486, we successfully antagonized the P receptor-mediated effects without interfering with either glucocorticoid or androgen receptor-mediated mechanisms. These findings indicate that progestins, rather than P metabolites, are influencing the observed behavioral responses. Furthermore, the RU486 antagonism of sexual behavior in our study suggests that the nuclear progesterone receptor, and not some other receptor, is involved in these P-dependent behavioral responses. Other behaviors, such as autogrooming, were unaffected, suggesting that the behavioral effects of P receptor antagonism were specific to copulatory interactions.

A significant number of P receptors are expressed in both females and males in diencephalic nuclei, such as the MPOA and hypothalamus (5). No sex differences exist in either P receptor distribution (17) or concentration (5) in the MPOA, although differences have been reported in the VMN and arcuate nucleus (5,18). Experiments in male rats have shown that P is secreted by the adrenals and testes in a circadian rhythm, with peak levels being five times greater than those observed during nadir periods (16). It appears that peak P levels coincide with the onset of the

dark phase of the photoperiod, when typical sexual activity is most intense in males. This was the time when our males were tested. In view of the facts that masculine sexual behavior is regualted by the MPOA, an area rich in P receptors, and that there is a circadian pattern of P secretion in the male, the physiological role of P in mediating androgen-dependent reproductive behavior in males requires critical attention.

In summmary, the above data provide evidence that at physiological levels P may play a significant role in the normal expression of androgen-dependent sexual behavior in male reptiles and mammals. Clearly, more extensive studies must be conducted to delineate both the genomic and nongenomic neural mechanisms underlying the effects of progesterone in the male.

ACKNOWLEDGEMENTS

We thank C. Ron Harbaugh for his technical assistance and concealment of the capsule contents until the close of the experiment. We are also grateful for the editorial comments of Dr. Christopher Flores. This work was supported in part by an NSF predoctoral fellowship and an NIMH training grant awarded to L.J.Y., and a Research Scientist Award (0013) and NIMH grant (R37 41770) awarded to D.C.

REFERENCES

1. Albin, J.; Vittek, J.; Gordon, G.; Altman, K.; Olivo, J.; Southern, L. On the mechanism of the anti-androgenic effect of medroxyprogesterone acetate. Endocrinology 93:417–422; 1973.
2. Bonsall, R.; Rees, H.; Michael, R. Uptake of medroxyprogesterone acetate by progestin and androgen target neurons in the brain and pituitary gland of male cynomologus monkeys. Neuroendocrinology 52:573–580; 1990.
3. Bottoni, L.; Lucini, V.; Massa, R. Effect of progesterone on the sexual behavior of the male Japanese quail. Gen. Comp. Endocrinol. 57:345–351; 1985.
4. Bradford, J. M. W. Treatment of sexual offenders with cyproterone acetate. In: Sitsen, J. M. A., eds. Handbook of sexology. vol. 6: The pharmacology and endocrinology of sexual function. New York: Elsevier Science Publishers; 1988:526–536.
5. Brown, T. J.; Clark, A. S.; MacLusky, N. J. Regional sex differences in progestin receptor induction in the rat hypothalamus: Effect of various doses of estradiol benzoate. J. Neurosci. 7:2529–2536; 1987.
6. Coirini, H.; McEwen, B. S. Progestin receptor induction and sexual behavior by estradiol treatment in male and female rats. J. Neuroendocrinol. 2:468–472; 1990.
7. Connolly, P. B.; Resko, J. A. Progestins affect reproductive behavior and androgen receptor dynamics in male guinea pig brain. Brain Res. 305:312–316; 1989.
8. Delville, Y. Progesterone-facilitated sexual receptivity: A review of arguments supporting a nongenomic mechanism. Neurosci. Biobehav. Rev. 15:407–414; 1991.
9. Erickson, C. J.; Bruder, R. H.; Komisaruk, B. R.; Lehrman, D. S. Selective inhibition by progesterone of androgen-induced behavior in male ring doves (*Streptopelia risoria*). Endocrinology 81:39–44; 1967.
10. Erpino, M. J. Hormonal control of courtship behaviour in the pigeon (*Columba livia*). Anim. Behav. 17:401–405; 1969.
11. Erpino, M. J. Temporary inhibition by progesterone of sexual behavior in intact male mice. Horm. Behav. 4:335–339; 1973.
12. Fadem, B.; Barfield, R.; Whalen, R. Dose–response and time–response relationships between progesterone and the display of patterns of receptive and proceptive behavior in the female rat. Horm. Behav. 13:40–48; 1979.
13. Frye, C. A.; Mermelstein, P. G.; DeBold, J. F. Evidence for a nongenomic action of progestins on sexual receptivity in hamster ventral tegmental area but not hypothalamus. Brain Res. 578:87–93; 1992.
14. Gibori, G.; Antczak, E.; Rothchild, I. The role of estrogen in the regulation of luteal progesterone secretion in th rat after day 12 of pregnancy. Endocrinology 100:1483; 1977.
15. Grassman, M.; Crews, D. Progesterone induction of pseudocopulatory behavior and stimulus-response complementarity in an all-female lizard species. Horm. Behav. 20:327–335; 1986.
16. Kalra, P. S.; Kalra, S. P. Circadian periodicities of serum androgens, progesterone, gonadotropins and luteinizing hormone-releasing hormone in male rats: The effects of hypothalamic deafferentation, castration and adrenalectomy. Endocrinology 101:1821–1827; 1977.
17. Lauber, A.; Romano, G.; Pfaff, D. Gene expression for estrogen and progesterone receptor mRNAs in rat brain and possible relations to sexually dimorphic functions. J. Steroid Biochem. Mol. Biol. 40:53–62; 1991a.
18. Lauber, A. H.; Romano, G. J.; Pfaff, D. W. Sex difference in estradiol regulation of progestin receptor mRNA in rat mediobasal hypothalamus as demonstrated by in situ hybridization. Neuroendocrinology 53:608–613; 1991b.
19. Lee, W.-S.; Smith, M. S.; Hoffman, G. E. Progesterone enhances the surge of luteinizing hormone by increasing the activation of luteinizing hormone-releasing hormone neurons. Endocrinology 127:2604–2606; 1990.
20. Lehne, G. K. Treatment of sex offenders with medroxyprogesterone acetate. In: Sitsen, J. M. A., ed. Handbook of sexology. vol. 6: The pharmacology and endocrinology of sexual function. New York: Elsevier Science Publishers; 1988:516–525.
21. Lindzey, J.; Crews, D. Hormonal control of courtship and copulatory behavior in male *Cnemidophorus inornatus*, a direct sexual ancestor of a unisexual, parthenogenetic lizard. Gen. Comp. Endocrinol. 64:411–418; 1986.
22. Lindzey, J.; Crews, D. Effects of progestins on sexual behaviour in castrated lizards (*Cnemidophorus inornatus*). J. Endocrinol. 119:265–273; 1988.
23. Lisciotto, C. A.; DeBold, J. F. Intravenous adminstration of progesterone and the onset of receptivity in female hamsters. Physiol. Behav. 49:679–683; 1991.
24. Martini, L. The 5 alpha-reduction of testosterone in the neuroendocrine structures. Biochemical and physiological implications. Endocr. Rev. 3:1–25; 1982.
25. Moguilewsky, M.; Philibert, D. Biochemical profile of RU 486. In: Baulier, E., ed. The antiprogestin steroid RU 486 and human fertility control. New York: Plenum Publishing Corporation; 1985:87–97.
26. Pfaff, D.; Schwartz–Giblin, S. Cellular mechanisms of female reproductive behaviors. In: Knobil, E.; Neill, J., eds. The physiology of reproduction. vol. 2. New York: Raven Press, Ltd.; 1988:1487.
27. Philibert, D.; Costerousse, G.; Gaillard–Moguilewsky, M.; et al. From RU 38486 towards dissociated antiglucocorticoid and antiprogesterone. In: Agarwal, M. K., ed. Antihormones in health and disease. vol. 19: Basel: Karger; 1991:1–17.
28. Philibert, D.; Moguilewsky, M.; Mary, I.; et al. Pharmacological profile of RU 486 in animals. In: Baulier, E., ed. The antiprogestin steroid RU 486 and human fertility control. New York: Plenum Publishing Corporation; 1985:49–68.
29. Piva, F.; Gagliano, P.; Motta, M.; Martini, L. Adrenal progesterone: Factors controlling its secretion. Endocrinology 93:1178–1184; 1973.
30. Roselli, C.; Horton, L.; Resko, J. Distribution and regulation of aromatase activity in the rat hypothalamus and limbic system. Endocrinology 117:2471–2477; 1985.
31. Sachs, B. D.; Meisel, R. L. The physiology of male sexual behavior. In: Knobil, E.; Neill, J., eds. The physiology of reproduction. vol. 2. New York: Raven Press, Ltd.; 1988:1393–1485.
32. Sar, M.; Stumpf, W. E. Cellular localization of progestin and estrogen in guinea pig hypothalamus by autoradiography. In: Stumpf, W. E.; Grant, L. D., eds. Anatomical neuroendocrinology. Basel: S. Karger A. G.; 1975:142–152.
33. Shughrue, P. J.; Stumpf, W. E.; Elger, W.; Schulze, P.-E.; Sar, M. Progestin receptor cells in the 8-day-old male and female mouse cerebral cortex: Autoradiographic evidence for a sexual dimorphism in target cell number. Endocrinology 128:87–95; 1991.

34. Stern, J.; Eisenfield, A. Distribution and metabolism of ^{3}H-testosterone in castrated male rats: Effects of cyproterone, progesterone and unlabeled testosterone. Endocrinology 88:1117–1125; 1971.
35. Tennent, B. J.; Smith, E. R.; Davidson, J. M. The effects of estrogen and progesterone on female rat proceptive behavior. Horm. Behav. 14:65–75; 1980.
36. Thornton, J. E.; Nock, B.; Feder, H. H.; McEwen, B. S. Sex differences in cytosolic progestin receptors in microdissected regions of the hypothalamus/preoptic area of guinea pigs. Brain Res. 503:253–257; 1989.
37. Warembourg, M.; Poulain, P.; Jolivet, A. Progesterone receptor-containing neurons in the guinea-pig mediobasal hypothalamus have axonal projections to the medial preoptic area. J. Neuroendocrinol. 4:273–279; 1992.
38. Young, L.; Greenberg, N.; Crews, D. The effects of progesterone on sexual behavior in male green anole lizards (*Anolis carolinensis*). Horm. Behav. 25:477–488; 1991.
39. Young, L.; Lopreato, G.; Horan, K.; Crews, D. Cloning and in situ hybridization analysis of estrogen receptor, progesterone receptor and androgen receptor expression in the brains of unisexual (*Cnemidophorus uniparens*) and sexual (*Cnemidophorus inornatus*) whip-tail lizards. J. Comp. Neurol. 347:288–300; 1994.