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STEROID REGULATION OF SEXUAL BEHAVIOR

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SUMMARY

Experimental data on rats and rabbits are reviewed, in support of the idea that estrogen is involved in the control both of male and female sexual behavior in these species. Female sexual behavior is stimulated by estrogen alone or estrogen in combination with progesterone. It can, however, also be induced by aromatizable, but not by non-aromatizable, androgens. Masculine sexual behavior is stimulated by aromatizable androgens or by non-aromatizable androgens combined with estrogen. The stimulatory effect of androgen on masculine sexual behavior is blocked by some aromatization blockers. This blocking effect can be reversed by estrogen. It is suggested that under normal conditions estrogens and androgens interact in producing sexual behavior.

INTRODUCTION

In the present paper we will discuss some data, obtained in our laboratories, related to the nature of the hormonal stimulus involved in the control of subprimate mammalian sexual behavior.

Female sexual behavior

Estrogen stimulates lordosis behavior in females of all subprimate species tested [for review see 1]. Some species require only estrogen for the display of sexual behavior while others need the sequential action of estrogen and progesterone (P). Species like the rat and guinea pig, requiring both estrogen and P, have short periods of estrous or heat, and are sexually non-receptive throughout the major part of the cycle. Other species, like the cat[2] and the rabbit[3], requiring only estrogen, show prolonged periods of estrous, during which they accept the male. Surprisingly, only a few studies comparing the efficiency of various estrogens in inducing estrous have been reported. Table 1 shows the potency of four natural estrogens in inducing estrous behavior of ovariectomized rats[4]. Estradiol (E_2) was the most potent estrogen in eliciting estrous, followed by estrone (E_1), estriol (E_3), and estrone sulphate. A similar rank of potency for natural estrogens was found in the ovariectomized guinea pig[5].

The stimulating effect of androgen on female sexual behavior has been observed in all primate and subprimate mammalian species tested[1–12]. It is of course possible that these androgen effects were due to brain aromatization which has been demonstrated both in primates[9] and subprimates[10]. Testing the effects of several aromatizable and non-aromatizable androgens on the sexual behavior of ovariectomized rabbits and rats, we found that aromatizable androgens (testosterone (T), androstenedione, 19-hydroxyandrostenedione, dehydroepiandrosterone) induced lordosis behavior, whereas non-aromatizable androgens (dihydrotestosterone (DHT), androsterone) did not[13, 14]. This led us to propose that aromatization was essential for androgens to stimulate lordosis behavior. This hypothesis was supported by the observation that some antiestrogens (MER-25, CI-628) interfered with T-induced lordosis behavior in ovariectomized rabbits[15] and rats[16, 17]. The hypothesis is also consistent with the absence of nuc-

Table 1. Effect of diverse estrogens on estrous behavior and uterine weight in the rat

Group	Treatment (10 days)	No. of rats	Receptivity LQ[a]	% of animals receptive	Uterine wt
1	Sesame oil	8	0.027 L/M 2/74	13	105.7 ± 31.3
2	Estradiol (1 μg/day)	8	0.430 * 34/79	63	223.2 ± 30.4*
3	Estradiol (4 μg/day)	8	0.557 * 39/70	100	267.7 ± 51.5*
4	Estrone (1 μg/day)	8	0.187 * 15.80	50	212.1 ± 32.2*
5	Estrone (4 μg/day)	7	0.661 * 41/62	88	295.7 ± 63.1*
6	Estriol (1 μg/day)	7	0.043 3/70	29	171.2 ± 14.3*
7	Estriol (4 μg/day)	8	0.280 * 21/75	75	149.6 ± 16.0*
8	Estrone-3-sulfate (1 μg/day)	8	0.212 * 17/80	50	138.7 ± 45.8
9	Estrone-3-sulfate (4 μg/day)	8	0.183 * 13/71	50	152.0 ± 30.0

* $P < 0.01$ compared with control.
[a]LQ was calculated considering all rats including those which did not show lordosis.

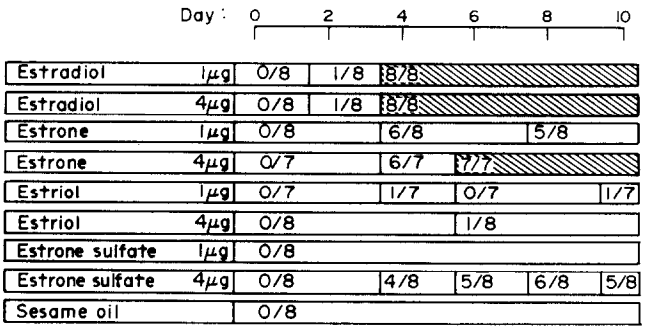

Fig. 1. Vaginal smears: Number of rats in estrus.

lear receptors for T in the female rat brain[10, 18, 19], and the occurrence of peripheral and central aromatization of androstenedione and T[20, 21].

Male sexual behavior

It is generally agreed that the display of masculine sexual behavior depends on androgen. Figure 2 shows the effect of 11 different androgens on the sexual behavior of castrated male rats[22]. Only T, androstenediol and androstenedione were effective in initiating the complete copulatory pattern. The effectiveness of these androgens was not correlated with their potency to stimulate the growth of the accessory sex organs. The regulation of the somatic and behavioral actions of T thus appears to involve different metabolic pathways and receptor systems[22, 24]. Interestingly, androstenediol was as effective as T in stimulating mounting behavior but not in stimulating ejaculatory behavior[23]. The finding that only aromatizable androgens stimulated mating suggest that aromatization is involved also in the control of male sexual behavior. Additional support of this idea is given by the finding that 19-OH-testosterone or 19-OH-androstenedione, representing intermediate compounds in the aromatization of T and androstanedione respectively, are effective in restoring copula-

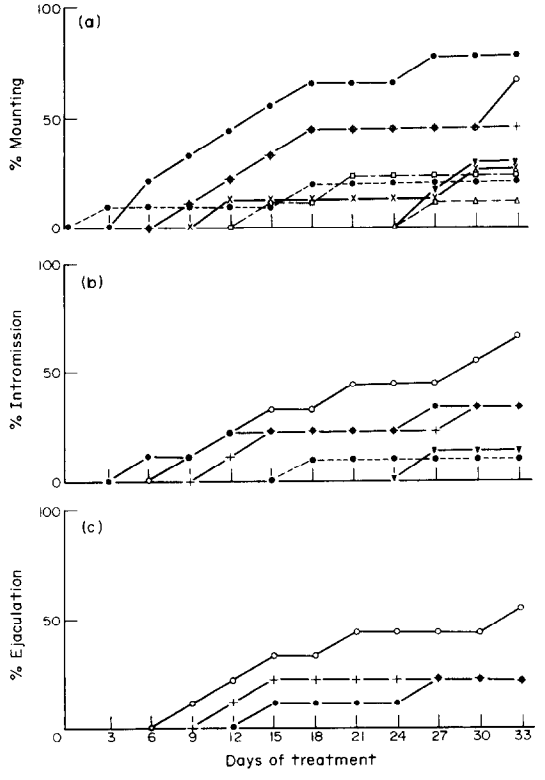

Fig. 2. First day of occurrence of either mount, intromission, or ejaculation response in rats receiving various androgen treatments. The data are shown as cumulative percentage of animals responding each test day in each group. Testosterone (—○—); androstenediol (— + —); androstenedione (—●—); dehydroepiandrosterone (--●--); 5α-androstanediol and oil (--×--); dihydrotesterone (—□—); 5β-androstanediol and androstanedione (--△--); 11β-hydroxyandrostenedione (—▼—).

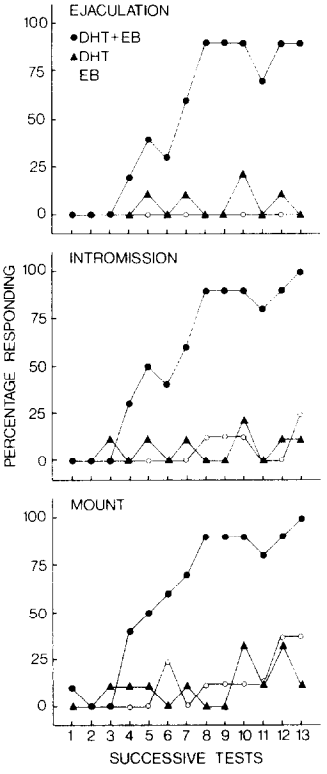

Fig. 3. Proportion of male rats in three treatment groups showing at least one mount, intromission or ejaculation during successive tests for sexual behavior. ●, Group A, male rats treated with dihydrotestosterone (DHT) and oestradiol benzoate (EB); ○, Group B, treated with EB; ▲, Group C, treated with DHT.

tory behavior in castrated rats[24, 25]. Qualitative differences between aromatizable and non-aromatizable androgens in their effectiveness to activate masculine sexual behavior were also found in hamsters[26] and in CD-1 mice[27]. Although in all species, except for the guinea pig, T was more potent in eliciting sexual behavior than its 5α-reduced metabolites, non-aromatizable DHT may induce sexual behavior in castrated male rabbits[28 but see 29], Swiss Webster mice[27], rhesus monkeys[30] and guinea pigs[31].

These results suggest that, at least in some species, aromatization is normally involved in the activation of masculine sexual behavior. Estrogen alone may initiate and restore full masculine copulatory behavior, when administered in relatively high dosages[32–39]. However, in sexually experienced castrated rats[40] and mice[41] even a small dosage as 1 μg estradiol benzoate (E_2B) restored full copulatory behavior. This was true even if the males were adrenalectomized[41]. It is of interest that females of many species display part of the male copulatory pattern and that both estrogen and some androgens may stimulate this behavior. Thus, in the ovariectomized rabbit and rat, administration of low dosages of E_2B (1 μg/day) elicited mounting behavior in almost half of the subjects[3, 42, 43], and some female rats treated with E_2B even showed ejaculatory behavior[44].

The possibility that estrogen synergizes with androgen in stimulating copulatory behavior, is supported by experiments in which E_2 combined with behaviorally ineffective androgens (DHT, fluoxymesterone, mesterolone) elicited complete sexual behavior in the castrated rat[38, 39, 45, 46], rabbit[47 but see 29] and CD-1 mouse[41]. Interestingly, E_1 as well as E_3 synergize with DHT in eliciting copulatory behavior in castrated male rats[48]. Synergism of estrogen and T in activating male copulatory behavior was also observed in pigs[49, 50] and in men[51].

In an attempt to evaluate the importance of aromatization, and therefore estrogen, for the expression of male sexual behavior, we studied the effect of antiestrogens on T-induced sexual behavior in castrated rats. As shown by Fig. 4, both MER-25 and cis-clomi-

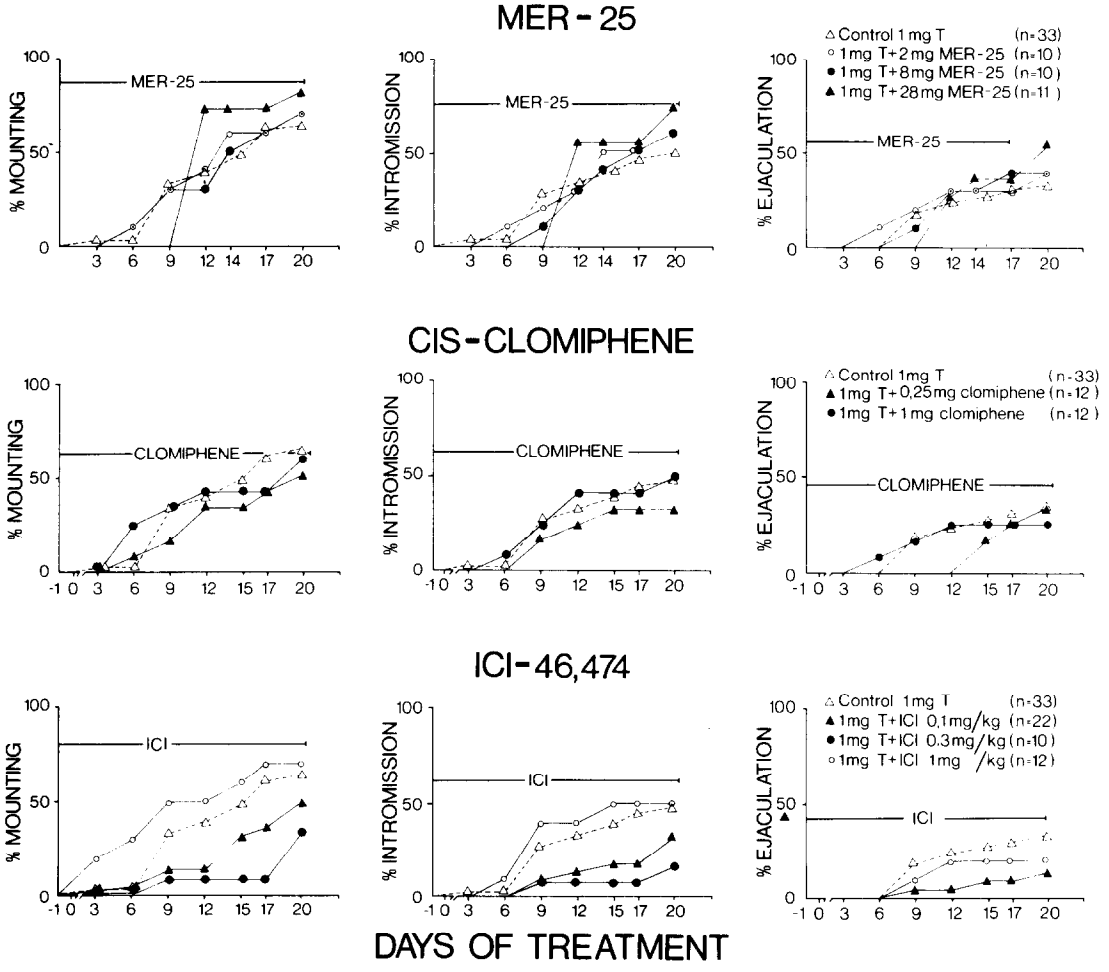

Fig. 4. Effect of antiestrogens (MER-25, cis-clomiphene and ICI-46474) on the response to T administration. Proportion of rats treated with androgen and antiestrogens displaying at least one mount, intromission or ejaculation. Androgen and antiestrogens were administered for the period indicated by the horizontal line.

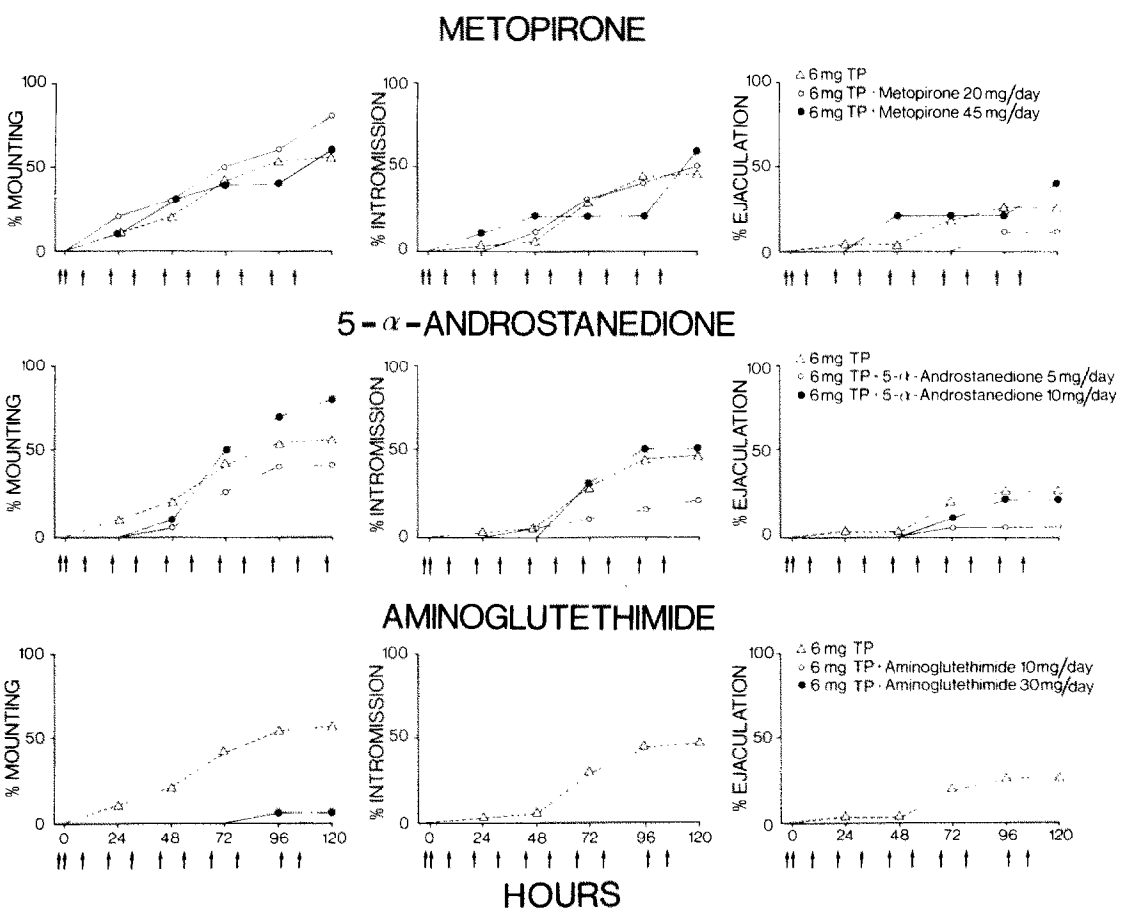

Fig. 5. Effect of some aromatase blockers on the response to a single injection of 6 mg TP. Thick arrow at 0 h indicates TP injection. Fine arrows indicate aromatase inhibitors injections. Note that aminoglutethimide administration abolished the response to TP.

phene failed to block the behavioral effect of T. Even in a high dose, ICI-46474 had only a weak inhibitory effect[52]. Failure of CI-628 to counteract androgen induced masculine sexual behavior in the rat has been reported[16], although, according to Luttge[53], a higher dose of CI-628 than that previously used to block lordosis[16] inhibited T-activated sex behavior in castrated rats.

Administration of MER-25 to ovariectomized female rabbits receiving either E_2B or TP, failed to block pseudomale behavior, while inhibiting lordosis[15]. Similar results were obtained in the ovariectomized rat[17].

The failure of some antiestrogens to block masculine sexual behavior in males and females, led us to study the effect of these compounds on the synergistic effect of DHT and E_2B. Surprisingly, MER-25 or cis-clomiphene did not interfere with the intense behavioral response induced by E_2B combined with DHT. These results indicate that the stimulatory effect of estrogen on masculine sexual behavior is not mediated by receptors having affinity for either MER-25 or cis-clomiphene, and that these receptors differ from those involved in lordosis behavior.

In recent years, several compounds of various chemical characteristics, have been reported to interfere with androgen aromatization *in vitro* and *in vivo*[54, 55]. We therefore decided to analyze the effects of some of these compounds on the behavioral response to T. Figure 5 shows the effect of three proposed aromatization blockers (metopirone, 5α-androstanedione and aminoglutethimide) on the response to T. Neither metopirone nor 5α-androstanedione interfered with the behavioral response. However, aminoglutethimide suppressed the sexual behavior. Similar inhibition was more recently obtained with 4-OH-androstanedione and androstantrienedione (unpublished data). Aromatization blockers are known to interfere with other biochemical processes, and therefore the inhibitory effect of these compounds may be unspecific. However, the fact that aminoglutethimide failed to block the stimulatory effect of DHT combined with E_2B on masculine sexual behavior rules out that possibility. Furthermore, the inhibiting effect of aminoglutethimide on T-induced sexual behavior in the rat was prevented by E_2B.

As in the case of the female, a series of biochemical studies support the idea that estrogen participates in the induction of masculine sexual behavior. Thus, androgen aromatization has been reported to occur

in male brain tissue, particularly in areas presumably involved in the regulation of sexual behavior[9, 56]. Lieberburg and McEwen[57] recently demonstrated that the main nuclear metabolite of T in the hypothalamus and preoptic area of the male rat is E_2. Moreover, antiandrogens such as cyproterone acetate and flutamide, do not block sexual behavior in the intact or castrated T-treated male rat[58–60].

In summary, it appears that, at subprimate level, estrogen is involved in the activation both of feminine and masculine sexual behavior. In females of some species, particularly those with long estrous cycles, estrogen alone stimulates feminine sexual behavior while in others estrogen combines with progesterone to produce full estrous behavior. Estrogen alone or aromatizable androgens with weak androgenicity such as androstenediol and 19-OH-testosterone are capable of maintaining such parameters of masculine sexual behavior as mounting, and may influence or shorten the latency of masculine copulatory responses.

Unless combined with estrogen, non-aromatizable androgens, such as dihydrotestosterone are almost completely ineffective in stimulating sexual behavior. A role of aromatization in androgen-induced feminine and masculine sexual behavior is further supported by the inhibitory effects exerted by some aromatization inhibitors on masculine sexual behavior (aminoglutethimide, androstatriendione and 4-OH-androstenedione).

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DISCUSSION

Grant. Considering these neuro-endocrinological studies, I would like to have more investigation of a hormone which is very neglected, that is dehydroepiandrosterone sulphate. About 20 years ago, G. Pincus and his colleagues at the Worcester foundation showed that dehydroepiandrosterone and its sulphate were among the best of the steroids for protection against sertonin antagonists. I wonder if you have any ideas about DHA?

Beyer. We have tested dehydroepiandrosterone on female sexual behaviour and we have found that dehydroepiandrosterone elicits lordosis behaviour in ovariectomized rabbits (Beyer C., Vidal N. and Mijares A.: *Endocrinology* **87** (1970) 1386–1389). But we have not tested dehydroepiandrosterone sulphate. I agree that it would be extremely important considering the high concentration that you find in plasma.

Taylor. We have been studying the effects of sex hormones and oral contraceptives on gastric secretion in cats (Albinus *et al. J. Endocr.* **69** (1976) 449), and as a side-line to this investigation we observed some quite remarkable changes in the sexual behaviour of the animals. Some of these changes were quite bizarre and unexpected. When male cats were given ethynyl oestradiol they became highly feminised and docile, as one might expect, and untreated male cats attempted to mate with them. Conversely, female cats treated with methyl testosterone became very aggressive and masculinised and attempted to mate with their untreated female cage companions. However, when male cats were treated with 'Minovlar' (ethynyl oestradiol plus norethisterone acetate) they adopted a female behaviour pattern. Untreated female cats in the same room as these treated males became sexually aroused and went into oestrus. When female cats were given 'Minovlar' they were highly sexually aroused and were wildly excited when untreated male cats were caged in the same room, and the male cats were also sexually aroused. For those of you who have observed the mating behaviour of a pair of cats, you can imagine the wild noises generated by six male and six female cats in the same room housed in separate cages! It seems to me that some sort of pheromonal influence was at work here, but I wonder what relationship these behaviour patterns have to receptor mechanisms in the brain. Is anything known about receptors in brain for ethynyl oestradiol and the synthetic progestin norethisterone acetate? I should add that the latter compound alone also induced these behavioural changes, although the effects were not so great as when oestrogen was also given.

Beyer. I believe that some implications regarding estrogen receptors can be done from our studies. For example, it is clear to me that the estrogen receptors involved in the activation of sexual behavior both in females and males must bind estriol besides estradiol and estrone. Another interesting deduction arising from our studies is that the estrogen receptors involved in female sexual behaviour differ from those involved in the induction of male sexual behaviour, since you can block estrogen induced female sexual behaviour by MER-25 or ICI-4.6.4.7.4. while you don't block male sexual behaviour using these antiestrogens.